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EXPERIMENTS ON YOUNG CHICK EMBRYOS CULTURED IN VITRO

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Cutting and deficiency experiments have been made on young chick embryos cultured *in vitro* according to the watch-glass method of Waddington.[4] The material consisted of chick blastoderms from an early primitive streak to the head process stages. Lately, because of certain difficulties, a medium consisting of agar, serum and chick embryo extract has been used. These experiments were begun in 1932 at the Strangeways Research Laboratory, Cambridge, England, while the writer held a C. R. B. Educational Foundation Fellowship for study in Belgium and have been continued since with financial assistance from the National Research Council.

Although not a common phenomenon in the development of the chick, embryos are occasionally found which show various degrees of duplication of the axial structures. Undoubtedly this occurs very early due to interference in developmental processes. Double or partially double embryos are known among mammals and have been produced experimentally in the amphibia. Interference with the formative processes whereby organs arise from paired rudiments, i.e., heart, will produce double organs. In order to test the capacity of these stages to give the duplication of axial structures, a median sagittal cut was made from the anterior part of the primitive streak to the germ wall. Such a cut is often extended farther posteriorly by mechanical factors.

Several different types of results were secured, depending apparently upon the way the cut was made. Double brains and anterior part of the neural tube were found but the medial sides had thinner walls or were incomplete, the divisional constrictions were faint and the eye was absent. One objection to this technique is the shortness of the culture period. Double but unequal notochords were found as well as irregular foreguts. Frequently both the nervous tube and foregut were incomplete or the folds widely separated. Double notochords were found only in the explants

from stages previous to that of the head process, and, where only one notochord was present, the side lacking this structure was more malformed. The two notochords were never of equal differentiation.

Certain cases gave more or less of a complete nervous system only on one side, the other consisted of a thickened layer of nervous tissue with or without eye material. The best case showed two irregular brains each with one eye, two unequal notochords, two incomplete foreguts and two sets of somites in the anterior end. Posteriorly the differentiation was quite typical. In another case the original cut had been extended posteriorly and two separate embryos were found each with its pairs of somites.

When the epiblast and hypoblast from the younger stages were cultured separately, these layers often grew as sheets of undifferentiated tissue. In other instances the epiblast gave nervous tissue, mesoderm and notochord. Environmental inhibition would account for this result since others have found by the method of chorioallantoic grafting that more differentiation will occur,[1] but in this case the culture period is longer. Somites appeared in explants of older stages but pulsating heart tissue was not seen. These observations confirm certain previous results of Waddington.[4]

In view of the importance attached to Hensen's node as a center of organization in the chick by certain investigators,[3,5] this structure, together with a variable amount of the node field, was removed and cultured to one side on the same mass of medium. Generally the deficient region was replaced by undifferentiated growth, although in some cases regeneration seemed to have occurred and nervous tissue, mesoderm and endoderm could be identified. With a few exceptions the various stages tested showed quite a normal development, for this environment, anterior to the deficient region. The heart was frequently paired.

Posterior to this region many cases showed the presence of neural fold or tube formation and also of somites. If the piece removed was too large, the differentiation of the posterior end was frequently destroyed, inhibited or malformed by environmental mechanical forces. Splitting along the primitive streak often occurred. Gut formation was generally abnormal because of interference with the formation of the body folds. Notochord tissue was not found. The isolated piece containing Hensen's node and the node field gave nervous tissue, pulsating heart tissue and a few somites.

When the young head process stage was cut transversely into 3 pieces, one cut anterior to the node and the other so as to bisect the streak, the anterior piece gave brain and gut. The middle third gave brain, neural tube, somites, paired hearts, notochord and gut. The posterior third gave medullary plate or folds and a few somites. In the latter the nervous plate was frequently split or the somites lacking. If a piece of the primitive streak region is removed posterior to the node, posterior axial structures differentiate. When the area removed is small, the neural folds extend

around the deficient region. In the posterior region the neural folds are often widely separated or split apart. When the same type of cut is made with the medium primitive streak stage, neural folds and somites appear posterior to the cut. No notochord was found in any explant from which Hensen's node had been removed.

Evidence has been presented[3,5] to show that the node acts as an organizer during development and is concerned in the posterior differentiation of the axial structures in the chick. Others[7,8] failed to secure posterior differentiation following the isolation or destruction of the node. On the other hand there is evidence[1,2,4,6] which seems to indicate that destruction, removal or isolation of the node from the streak does not necessarily inhibit the appearance of axial structures in areas around or posterior to it. For a discussion of this subject reference should be made to a recent paper by Dalton.[1]

Extracts of the thyroid gland and the growth promoting principle of the pituitary, when added to the culture medium either singly or in combination, failed to produce any perceptible effect on the growth or differentiation. Either the embryo was not able to utilize this material in the way it was given or the culture period, 48–72 hours, was too short for any effect to appear.

The results obtained in these studies indicate that (1) duplication of a kind may take place in the case of certain axial structures even in stages as late as those tested; (2) the isolated hypoblast does not differentiate alone while the epiblast may give nervous tissue, notochord, mesoderm and somites; (3) the differentiation of posterior axial structures, i.e., nerve cord and somites, may occur in young blastoderms from which Hensen's node together with more or less of the node field have been removed, but the notochord is lacking; (4) the addition of thyroid extract and the growth promoting principle of the pituitary to the culture medium do not cause any perceptible effect on the development of the explant.

[1] Dalton, A. J., *Jour. Exp. Zoöl.*, **71**, 17 (1935).

[2] Hoadley, L., *Arch. Biol.*, **36**, 225 (1926).

[3] Hunt, T. E., *Jour. Exp. Zoöl.*, **59**, 395 (1931); *Proc. Soc. Exp. Biol. Med.*, **28**, 626 (1931); *Anat. Rec.*, **55**, 41 (1932).

[4] Waddington, C. H., *Phil. Trans. Roy. Soc. London*, Series **B221**, 179 (1932); *Roux' Arch.*, **128**, 502 (1933).

[5] Willier, B. H., and Rawles, M. E., *Jour. Exp. Zoöl.*, **59**, 429 (1931).

[6] Kopsch, F., *Zeit. Mikro.-anat. Forschg.*, **35**, 254 (1934).

[7] Wetzel, R., *Ergänzungsheft z. Anat. Anz.*, **60**, 127 (1925); *Roux' Arch.*, **106**, 463 (1925); *Ibid.*, **119**, 188 (1929).

[8] Dantschakoff, V., *Roux' Arch.*, **127**, 542 (1932).

A NEW PREHISTORIC CULTURE IN HAITI

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Archaeological investigations in the region of Fort Liberté Bay, North Haiti, have disclosed certain shell heaps or kitchen middens which contain numerous secondarily chipped flint implements and no ceramic objects. These middens standing in sharp contrast to middens previously excavated in the West Indies, which normally contain pottery and no flint tools, clearly represent the remains of a distinct prehistoric culture. Non-pottery-bearing deposits have been found in Cuba, Santo Domingo and the Virgin Islands, representing what has long been thought to be a primitive, early culture in the West Indies, but flint tools have not been found in these or in any of the usual pottery-producing deposits. Since the technique of pottery manufacture was known throughout all the islands of the Antilles at the time of the conquest, the non-pottery-producing, flint-bearing deposits near Fort Liberté Bay may be interpreted as the remains of an early prehistoric culture preceding the pottery-making cultures that extended over the West Indies at the time of the discovery.

The first indication of the existence of this early culture in Haiti was observed in the large collections of archaeological specimens owned by Mr. and Mrs. Robert Pettigrew of the Haitian American Development Corporation. The many well-worked flint implements in this collection were brought in by natives who said they were found in certain sections of the plantation. In the spring of 1935 Mr. Allison V. Armour made it possible for me and Mr. B. Irving Rouse to undertake extensive investigations in north Haiti under the auspices of Peabody Museum, Yale University. Assisted by Mr. and Mrs. Pettigrew and the Haitians who had collected for them, we were able to locate five aboriginal dwelling sites which produced these flint implements.

Flint-Bearing Deposits.—Adopting the names of the old French colonial land grants, we have termed the flint-producing sites Couri No. 1, Couri No. 2, La Rivière Maurice, Savanne Carrée No. 1 and Savanne Carrée No. 2. All are within three miles of the bay but none is actually on the shore. Four of the sites are marked by shallow marine shell deposits never over forty centimeters in depth. The fifth, Couri No. 2, is a low mound one meter in height and approximately thirty meters in diameter. The composition of all five deposits is relatively homogeneous. Marine shells are scattered through black sandy soil which contains a large quantity of wood ash and charcoal. No bone refuse of any kind appears. All artifacts found throughout the deposits are made of stone, with the single

exception of a shell ornament curiously resembling a "fleur-de-lis." This was found near the surface at Savanne Carrée No. 1.

The majority of the artifacts found were complete or fragmentary implements made of flint or of material which could be worked into tools with the chipping or flaking process employed in the manufacture of flint implements. In order to simplify terminology, all objects of this kind will be referred to here as flint. These tools may be classed as scrapers, knives and spear-heads. The scrapers are rough flakes two or three inches in diameter and of varying shapes, with at least one side worked down by the secondary chipping process to form a relatively sharp cutting or scraping edge. The knives are rectangular implements varying from four to ten inches in length, one to three inches in width and one-fourth to one inch in thickness. Some of the knives are sharpened by the chipping process on one edge and others on both edges. All appear to have been struck from a prepared block of flint, the line of cleavage still remaining on both surfaces, but the edges having been retouched. The spear-heads are dagger-shaped implements with a well-formed stem or hilt and a sharp leaf-shaped blade. They are, apparently, long thin flakes struck from a core, which have been formed by retouching the edges. Facets of the flaking process never extend across the flat surfaces as on the flint spear-heads found in North America. They vary in length from six to ten inches and in width from two to four inches. The technique of chipping indicated by the form of all these flint tools appears to be very simple and crude, contrasting markedly with the chipping technique evidenced by the usual type of flint implement found in the Americas.

Associated with the flint tools were found numerous grinding stones which are well-formed objects made of rough granular stone. The majority have two flat grinding surfaces. In outline some are oval, some rectangular and others round. All are small enough to be grasped conveniently in one hand. In addition, a few stone objects were found which may be termed double-headed axes or hammers. These implements are oval in outline and made from coarse-grained stones, apparently by the "pecking" process. The lateral sides are invariably notched as if the implements had been intended for hafting. In some cases both ends have been ground down to form relatively sharp cutting edges, while in other cases the ends are blunt and pitted although roughly worked to an edge. These objects vary from six to fourteen inches in length and from three to ten inches in width. The smaller, sharpened tools would serve very well as axes, but the large, blunt-edged specimens could not have been used as cutting tools.

Fragments of red ocher were also found throughout all flint-bearing

deposits. Two small tubular stone beads were encountered at the Couri No. 1 site.

Pottery-Bearing Deposits.—During the period of investigation in the region of Fort Liberté Bay we also excavated six large pottery-bearing shell midden sites which have been termed Macady, Carrier, Meillac, Diale No. 1, Diale No. 2 and Moyeaux. Two of these sites are marked by shallow refuse deposits never exceeding fifty cm. in depth, and four are composed of low mounds with an altitude of one to two meters. All of these pottery-bearing sites are within four miles of the bay shore and the two at Diale extend practically to the water's edge. The composition of all these refuse deposits is essentially the same, consisting of marine and terrestrial shells, blackened soil, ash, charcoal, bones of the manati, hutia, fish, birds and turtles, interspersed with numerous ceramic objects and polished stone and shell implements.

The pottery removed from the Carrier site and from the Diale No. 2 site was essentially the same, and stood in contrast to the pottery removed from the Meillac, Moyeaux, Nacady and Diale No. 1 sites. The difference between the pottery removed from these groups of middens indicated that we were dealing with two cultural types or two phases of the same culture pattern. The pottery from the Carrier site and from the Diale No. 2 site has been termed the "Carrier type," while that from Meillac, Macady, Moyeaux and Diale No. 1 has been termed the "Meillac type." The distinctions are primarily based on pottery ornamentation, the stone and shell implements found in all pottery-bearing sites being essentially the same. Briefly, the Carrier type is characterized by vessels decorated with elaborate incising and with a peculiar modeled head lug or "adorno," while the Meillac type is characterized by numerous boat-shaped bowls with loop handles, modeled head lugs of a much simpler form, and a rarity of line and puncture incision. Pottery from both types of sites is relatively crude and coarse in composition, and the distinction is based primarily on the technique of ornamentation which is more complex on pottery of the Carrier type.

Both types of pottery from the deposits in the Fort Liberté Bay region conform to the general pattern of cultural traits found in shell middens throughout the Greater Antilles and the Virgin Islands. This complex of traits I have referred to as the "Shell Culture," and it has been tentatively associated with the Arawak-speaking peoples occupying the Greater Antilles at the time of the conquest. (Rainey, "A New Prehistoric Culture in Puerto Rico," *Proc. Nat. Acad. Sci.*, **21**, No. 1, Jan., 1935.) This Shell Culture has been found in stratified deposits in Puerto Rico overlying an earlier trait complex referred to as the Crab Culture. No indications of this earlier Crab Culture were found in north Haiti.

A stratigraphic study of Shell Culture deposits in Puerto Rico suggested

an early and a late phase with the distinction based on more complex pottery ornamentation in the late phase. These two phases of the Shell Culture are apparently duplicated in the Shell Culture deposits in Haiti, with the Meillac type corresponding to the early phase and the Carrier type to the late phase. However, we were not fortunate enough to discover a stratified deposit in Haiti, and as yet there is no proof that the Carrier type succeeded the Meillac type. A careful analysis of the material has not yet been made and it is possible that some clue to the relative ages of these two Shell Culture types in Haiti may appear with an adequate study of the material.

Conclusions.—The result of the recent excavations in Haiti may be summarized as follows: five aboriginal dwelling sites producing flint tools, grinding stones and double-headed axes or hammers, but no pottery, represent a distinct, early, and as yet unrelated culture in the West Indies; six aboriginal dwelling sites producing abundant pottery, polished stone and shell tools, but no flint implements, represent a later culture which correlates with the Shell Culture pattern (probably Arawak) that has been found distributed over the Greater Antilles and the Virgin Islands; and finally, two types or phases of this later, Shell Culture, exist in Haiti with the relative ages still problematic.

In the report of the Puerto Rican research carried out in 1934 (unpublished) I have shown that the early, Crab Culture, in Puerto Rico is distributed from Trinidad through the Lesser Antilles to Puerto Rico, and that the later, Shell Culture, is distributed from the Virgin Islands through the Greater Antilles to the Caicos Islands in the Bahamas. Since the Crab Culture material lies below the Shell Culture in stratified deposits in Puerto Rico, I have assumed that early and late periods of migration into the West Indies are indicated. At present, northeastern South America appears to be the source of both these culture movements. In the preliminary report of the Puerto Rico work undertaken in the summer of 1934 (*Proc. Nat. Acad. Sci.*, **21**, No. 1), these two culture periods were supplemented by an hypothecated "Problematic Recent Culture" based on the presence of certain carved stone objects such as "collarstones," "three-pointed stones" and "stone masks" in numerous surface collections of Puerto Rican archaeology. None of these objects were found in the shell middens excavated in 1934. Additional work in Puerto Rico (1935) has produced fragments of some of these carved stone objects associated with Shell Culture artifacts in the shell middens, indicating that the existence of a "Recent Culture" is less likely.

Correlating the Puerto Rican work with that just completed in Haiti, a tentative sequence of culture horizons in the West Indies may be indicated as follows:

III. Shell Culture (probably Arawak)
Greater Antilles and the Virgin Islands.
In stratified deposits above the Crab Culture complex in Puerto Rico and demonstrably later than the Crab Culture complex in the Virgin Islands.
B—Late phase in Puerto Rico.
Possibly the "Carrier type" in north Haiti.
A—Early phase in Puerto Rico.
Possibly the "Meillac type" in north Haiti.
II. Crab Culture
Lesser Antilles and Puerto Rico.
In stratified deposits below the Shell Culture in Puerto Rico and demonstrably older than the Shell Culture in the Virgin Islands.
I. Flint Culture
North Haiti.
In non-pottery-producing, flint-bearing deposits, not in stratified sites, but always segregated and assumed to be older than all pottery-producing deposits.

Rainey, F., *Proc. Nat. Acad. Sci.*, **21**, 12–16 (1935).

SUMMARY OF INVESTIGATIONS OF VARIABLE STARS

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In the measurement of distances, both within and outside the galaxy, and in the investigation of stellar development, stars that vary in light continue to be useful and important. Much variable star work contributes only to knowledge of the individual stars themselves, but several of the investigations in progress at Harvard, such as those summarized below, are designed to make use of stellar variation as an instrument in the attack on problems of a more general nature. In such researches emphasis is placed either on extensive surveys that are aimed to provide material for the analysis of frequencies of types, luminosities and distances, and the study of distribution in space; or on the peculiar types and anomalous behaviors of variable stars.

In successive sections the following subjects are treated:

(1) Discovery and distribution of a thousand new variable stars in the Small Magellanic Cloud.

(2) The periods of super-giant Cepheids in the Small Magellanic Cloud.
(3) Three hundred new variable stars in low galactic latitudes.
(4) High latitude variable stars and the thickness of the galaxy.

1. *Variable Stars in the Small Magellanic Cloud.*—In 1904 and in the three or four following years Miss Leavitt carried through her historically important investigation of variable stars in the two Magellanic Clouds. For the study of the Small Cloud she had twenty long exposure photographs made with the 24-inch Bruce telescope at Arequipa.[1] A total of 992 stars were marked and published as variables, and subsequently periods and light curves for more than two hundred of them have been derived from Bruce plates by various workers at the Harvard Observatory.

Gradual improvement in photographic plates and observing technique has in recent years resulted in somewhat better photographs of the Small Cloud than were available to Miss Leavitt. A systematic reëxamination of the region has therefore been undertaken, and intercomparison of new and old plates has been made, chiefly by Miss S. F. Mussells, as follows:

PLATE	DATE	EXPOSURE (MINUTES)	COMPARISON
A 3393 (positive)	Nov. 10, 1898	300	A 14371
A 6984 (positive)	Oct. 3, 1904	120	A 14276, A 14876
A 14276	Oct. 8, 1929	150	A 6984
A 14371	Nov. 28, 1929	225	A 3393
A 14876	July 31, 1930	240	A 6984

The method of superimposing negative on positive was used. About thirteen hundred new variables were detected, and a large majority of Miss Leavitt's stars were rediscovered. From a study of the results in hand we estimate that stars for which the median magnitudes are below 16.5 are as yet very incompletely recorded, and therefore, since the distance modulus of the Cloud is fainter than the seventeenth magnitude, it is clear that the survey for variables has not yet included the cluster type Cepheids. Such stars must be detected and studied with the aid of the 60-inch reflector of the Boyden Station, or similar instruments.

Because of the difficulty of the work in crowded regions, a considerable number of stars marked are probably not variable. To test the frequency of spurious variables, we have measured 175 of the stars on twenty plates and find that seventy-five per cent of those recently marked are certainly variable through reasonably normal ranges (greater than half a magnitude). The number of false variables in the list by Miss Leavitt is relatively less—perhaps fifteen per cent. We therefore know about eighteen hundred variable stars in the Small Cloud. In a similar supplementary survey of the Large Cloud the number was increased from Miss Leavitt's eight hundred to approximately fourteen hundred.

For both Magellanic Clouds Miss Leavitt's earlier discoveries included

most of the very brightest variables, and also those of largest range; but early and late surveys extend down to the same magnitude, which may be taken as 16.8 in the median. The average photographic amplitude for the Leavitt variables is about 1.2 magnitudes and for the new discoveries just less than a magnitude.

Making use of a preliminary luminosity curve derived some years ago for all the stars to magnitude 18.0 in the Small Cloud,[2] we now compute that 2.5 per cent of the stars to magnitude 16.8 are variable stars, presumably almost wholly of the Cepheid class. The corresponding number for the Large Cloud is about 1.5 per cent. For both systems the relative number of Cepheid variables will undoubtedly be increased through the study of more long-exposure plates and the inclusion of smaller ranges. Probably the total number will also be greatly increased when Cepheids of fainter apparent magnitude are included.

I have been assisted in various parts of this investigation by Mrs. D. H. Leavens, Dr. R. K. Marshall, Miss Jenka Mohr and Mr. P. S. Riggs.

2. *A Further Study of the Period-Luminosity Relation.*—The number of variables in the Small Magellanic Cloud for which light curves and periods are known has been increased to more than 200 through a special study by Miss Hoffleit of the Cepheids with absolute photographic magnitudes brighter than −2.0. The investigation was designed to throw more light on the form of the period-luminosity curve for super-giant Cepheids. The material heretofore available in the Magellanic Clouds and in the Milky Way for the brighter part of the curve has been very meager. Nevertheless this preliminary correlation has of necessity been used in determining the distances of the nearer external galaxies with the aid of Cepheids of highest luminosity.

A study of the new periods and magnitudes determined by Miss Hoffleit for fifty-three Cepheids,[3] and of the periods previously determined for Cepheids in the Small Cloud by Leavitt, Mohr, Morse, Riggs, Sawyer, Shapely, Wilson and Yamamoto, all using the Harvard photographic material, leads to the following conclusions:

(*a*) The period-luminosity curve, as used for the past ten years, does not yet need alteration in form, nor is it yet necessary to change the distance modulus for the Small Magellanic Cloud from the current value of $m - M = 17.3$ (magnitudes uncorrected for space absorption).

(*b*) The observational scatter around the mean curve is greater than that indicated by the earlier observations, but we believe it is to be attributed chiefly to uncertainties of secondary magnitude sequences and to the difficulties of measurement in crowded regions, and only slightly, if at all, to local absorption in the Cloud. Something like five per cent of the investigated variable stars are superposed variables of the galactic system,

chiefly of the long period, W Ursae Majoris, and cluster types. This frequency of galactic variables is normal for a closely searched region in galactic latitude $-45°$.

(*c*) A difference in slope between the period-luminosity curves for the Small Cloud and the Large Cloud remains unexplained, but the puzzle may be resolved either by a comparable study of the super-giant Cepheids in the Large Cloud, or, since trouble may lie in the scales of apparent magnitudes, through an extended intercomparison of magnitude sequences for the two systems.

(*d*) The correlation, suggested by Lundmark, between the period and the amplitude of classical Cepheids is unimportant for the Small Cloud, according to the data assembled for two hundred variables.[4] The material does show, however, that for the Cepheid variables with periods between twenty and forty days the amplitudes on the average are thirty per cent greater than for stars with periods shorter or longer—a result in agreement with data on Cepheids of the Milky Way, and probably the chief basis for the suggestion of a period-amplitude relation. Since the spectra of the Cepheids with longer periods are of Class K and later, it is probable that absorption bands affect the ranges in photographic light. For all period lengths, from one day to a hundred days, there is a wide scattering in the values of the observed amplitudes. Most of the periods now known are less than twenty days; for them the average amplitude is one magnitude (photographic), with a mean deviation of two tenths of a magnitude.

(*e*) A disturbing lunar factor in the determination of the periods of classical Cepheids is noted by Miss Hoffleit in her study of the variables of the Small Cloud; its effect is analogous to the troublesome diurnal interruption of series of observations for cluster type Cepheids, and to the annual interruption in the observation of most long period variable stars. Because of the monthly interruption, due to full moonlight, of the photographic exposures on the Small Magellanic Cloud, four of the periods previously derived were erroneous, and the ratio of early to corrected period is one month divided by one month plus or minus the false period.

3. *Progress of Research on Faint Milky Way Variable Stars.*—The program for the investigation of Milky Way variable stars, first described in these PROCEEDINGS in 1928, has resulted in the completion recently of extensive studies in three of the Milky Way variable star fields—Nos. 168, 184 and 193—in which nearly 900 new variables have been recorded.[5] Studies of seven other low latitude fields were made previously by various workers in the Harvard Observatory, under the general supervision of Miss Henrietta Swope, and published in a series of Harvard Observatory Bulletins. These earlier papers dealt with more than 1100 variable stars.

The new results add to the rapidly increasing information concerning

individual variable stars of various types and to the knowledge of frequencies of types, magnitudes and periods in various parts of the Milky Way; but since the three fields lie in that part of the southern Milky Way that is much disturbed by obscuring nebulosity, the use of the material in mapping out galactic distances and structures is of restricted value until appropriate corrections are deduced for space absorption through studies of star colors or analyses of the distribution of external galaxies, or in other ways. In some regions, such as Milky Way Field 233, the study of which will be reported on elsewhere in the near future, the number of external galaxies observed affords assurance of essential clarity of space and thus permits the variable stars to be used successfully and without correction as distance indicators.

(*a*) Of the three new fields for which partial results can now be reported, the first (*MWF* 168) lies on the galactic circle at 13^h43^m, $-61°.1$. With the earlier completion of two neighboring fields—*MWF* 167 and 169—it is now possible to compare the numbers and magnitudes of variable stars in a strip of the sky twenty degrees long across the Milky Way circle. The number of variable stars per square degree is approximately the same for all three fields.[6] An unusually high proportion of the stars are eclipsing variables of short period.

(*b*) The investigation of *MWF* 184 (centered at 17^h32^m, $-42°.5$) results in 350 new variable stars. The region is not distant from the direction to the galactic center. Perhaps the most noteworthy result of the study is that the 166 variables of long period have a small dispersion in maximum photographic magnitude around the value 14.75. It is probable that most of these stars are associated with the rich star clouds at the center of the galactic system.

If we assume the central clouds to be 10,000 parsecs distant (as computed on dynamical grounds) and the average color index of the variables at maximum to be 1.5 magnitudes, we find that the mean absolute visual magnitudes are clustered about the value -1.75. This result falls within the range of the values recently compiled by Thackeray,[7] but near the brighter limit.

If we assume that there exists much space absorption between the earth and this low latitude field in Sagittarius, the deduced absolute magnitude for the longer period variables becomes abnormally bright. This fact may be taken to indicate a rather small amount of general or localized space absorption between us and the star cloud of *MWF* 184; but since no certainly recognized external galaxies are found within the bounds of the field, we must assume that beyond this central cloud there is strongly absorbing material. The variable star survey, of course, should be extended another magnitude or two before we conclude finally that the maximum frequency is at apparent photographic magnitude 14.75, as now ob-

served. Special observations are being made at the Boyden Station to test the long period variable stars in *MWF* 184 for color excess.

(*c*) Four hundred and fifty new variable stars, in addition to 158 already found and published, have now been discovered in *MWF* 193, at 18^h24^m, $-23°.3$, not far from the galactic center in Sagittarius. It is one of the richest variable star fields on record; the examination, which is not yet exhaustive to the magnitude limit 16.5, shows about ten variable stars per square degree. Of the new variables Miss Hoffleit has found the periods for sixteen, one of which is of the cluster type, one (H.V. 6696) is apparently a Cepheid variable of 66 days, and all the others are typical long period variable stars. Unless the suspected Cepheid is of peculiar intrinsic luminosity, or is heavily dimmed by space absorption, it must be a remarkably distant star; if it is normal and unobscured, the computed distance is nearly 100,000 parsecs.

4. *Variable Stars in High Galactic Latitudes.*—Through the study of the magnitudes of faint cluster type Cepheids in regions near the north and south galactic poles we are able to obtain a measure of the extent of the Milky Way system at right angles to its plane. The same type of star in galactic latitudes between $+20°$ and $+30°$, or $-20°$ and $-30°$, when discovered and studied with the most far reaching telescopic power available, will be able to tell us more about the extent of the Milky Way system along its plane than we are likely to obtain by photometric investigations near the galactic circle. The effectiveness of faint cluster type Cepheids in the measurement of galactic dimensions has increased considerably in recent years through the study of their absolute magnitudes, and through the investigations, at Harvard and at Mount Wilson, of the distribution of external galaxies, which show that in latitudes higher than 30° light absorption is not a serious hindrance to the photometric measurement of distances.

The discovery of cluster type Cepheids in high galactic latitudes was the principal aim of the systematic program inaugurated at both southern and northern stations of the Harvard Observatory some ten years ago. At that time the program was planned for the discovery of most of the variable stars to magnitudes 16.0 in seventy-two fields; a field of eighty square degrees is covered by the southern telescopes and of thirty-five square degrees or more by the northern instruments. Subsequently, the importance of the program led to the inclusion of the whole "high-galactic" sky in the systematic survey. The number of fields is thus increased by more than five times. Five different telescopes (three in the south and two in the north), in addition to the patrol cameras, are employed in this research.

Eleven of the high latitude fields have been studied in some detail,[8] and 374 variable stars have been found; in the same fields forty-three were already known. A preliminary examination of thirty-nine additional high

latitude fields, entailing the study of only five plates for each field, has yielded 556 new variable stars, of which forty per cent belong to the valuable cluster type class of Cepheids. Altogether the survey has added more than 300 cluster type variables to the 153 already known in latitudes higher than 20°. Many of these stars are very faint, the corresponding distances exceeding 10,000 parsecs. The use of such material in showing the great thickness of the Milky Way has been described in an earlier contribution to these PROCEEDINGS.[9] There is growing evidence that surrounding our discoidal galaxy, in analogy to the Andromeda Nebula, a more or less spherical "atmosphere" exists, composed of cluster type Cepheids and possibly of other objects characterized by high velocity.[10]

Many of the faintest variables in high galactic latitudes found on Harvard plates are of little use in the estimates of distance because they are dwarf eclipsing binaries of the W Ursae Majoris type. In the eleven high latitude fields we find the following distribution among types:

Cluster type Cepheids	38 per cent
Eclipsing variables	23
Classical Cepheids	0.4
Long period variables	12.3
Irregular variables	12.3
Periods or types unknown	14.0

The study of the high latitude variable stars obviously must proceed for several years before we can clearly indicate the outer limit of the galactic system.

[1] Leavitt, *H.A.*, **60**, No. 4 (1908); see also *H.C.* 79 (1904), and *H.C.* 173 (1912).
[2] Shapley, *H.C.* 260, 5 (1924).
[3] Hoffleit, *H.B.* 900 (1935).
[4] Lundmark, *Lund Medd.*, Ser. **I**, No. 128, 313 (1931); Hoffleit, *H.B.* 900 (1935).
[5] Shapley and Swope, *H.A.*, **90**, No. 5 (1934).
[6] *Ibid.*, p. 181.
[7] Thackeray, *The Observatory*, **58**, 288 (1935).
[8] Shapley and Hughes, *H.A.*, **90**, No. 4 (1934).
[9] These PROCEEDINGS, **19**, 29–34 (1933); Harvard Reprint 81.
[10] Shapley, *Mon. Not. R.A.S.*, **94**, No. 9, 791 (1934).

PRISMATIC DEVIATION AS A FUNCTION OF COSMICAL ORIENTATION

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Measurements of the total deviation of light in a rigidly constructed 2-prism astronomical spectrograph were made to detect any possible changes produced by aximuthal rotation of the instrument in its optical plane at various sidereal times. The refractive index was found to be constant within an accuracy of about 5×10^{-8}.

The spectrograph employed had been rigidly constructed for use at the Newtonian focus of the Wyeth 61-inch reflector. The two 65° prisms of Shott U. V. glass have a combined deviation of roughly 91° and a square aperture of $2^1/_2$ inches. With a camera of focal length 13 inches, a change of 8.0×10^{-7} in refractive index produces a displacement of one micron on the photographic plate. A grating with six clear spaces of 0.5 mm. width, centered 1 mm. apart, was ruled on a small mirror and used at the spectrograph slit to break the light into six 0.5 mm. lengths. The grating could be shifted once 0.5 mm. parallel to the spectrograph slit to obtain, by means of two successive exposures, nearly continuous spectral "lines" on the photographic plate.

The spectrograph was mounted upon, with its optical plane parallel to, the surface of a horizontal turntable. A mercury arc rested on the turntable in front of the spectrograph slit and grating, separated from the latter by a diffusing screen of opal glass. The experiment was carried out in the basement of a frame building with stone cellar walls, on the grounds of the Harvard Observatory.[1] The orientation of the apparatus was specified by the direction from which the light traveled in the air between the prisms. Seen from above, the light was always deviated in a clockwise sense. During any single exposure the grating occupied one of two positions, "up" or "down," before the vertical spectrograph slit. Eastman "33" plates were used and developed in *D*-11 developer.

A single exposure, with the slit up, produces on the photographic plate six segments of any spectral line. Another exposure, with the slit down, produces six complementary segments. If the index of refraction of the prisms does not change, then the two sets of segments will produce a complete spectral line. If, however, there is any difference between the refractive indices during the two exposures, then one set of segments will be displaced with respect to the other, in the direction of the dispersion on the photographic plate. Let half of the displacement be denoted by z. This quantity can be determined very accurately, without prejudice, as follows:

Choose an axis (the y axis) on the photographic plate, nearly parallel to the direction of the dispersion, and in the sense of increasing deviation. The x axis is to be nearly perpendicular to the y axis, on the plate. The equation of one set of segments (grating "up") is with sufficient accuracy

$$y = a + bx + cx^2 + z$$

and of the other (grating "down")

$$y = a + bx + cx^2 - z$$

These equations take into account the arbitrary origin of measurement, the deviation of the y axis from the axis of dispersion, and the curvature of the line, but assume the slit to be regular through second order terms. Choose twelve equally spaced values of x, each falling in one of the twelve segments, and measure the corresponding y's on a measuring engine. A least squares solution can then be made for z. The value of z is thus free of all errors arising from zero point, orientation and curvature of the spectral line (through second order terms), and also from deviations of the ways of the measuring engine from normality and linearity (through second order terms), but z is not free of small errors arising from irregularities of the slit. The mercury lines λλ3650, 3654 and 3663 were measured on the Coast and Geodetic Survey machine, and all measurements then reduced in the above manner.

Plates were exposed at six uniformly distributed times during the day, there being three plates at each time. There were four groups of two exposures each on a plate, the exposures of a single group combining to form an ordinary spectrum. Between the two exposures of a group the spectrograph was rotated, the grating shifted, but the plate held rigidly fixed in the spectrograph. Between groups, the position of the plate was, of course, changed. The sequence of exposures at each time was as follows:

First plate	*SuNd—NuSd—EdWu—WdEu*
Second plate	*SuWd—WuSd—NdEu—EdNu*
Third plate	*SdNu—NdSu—EuWd—WuEd*

Plates of the differents sorts are described by the series numbers 1, 2 and 3. Here the capital letters *S, N, E* and *W* denote the orientations South, North, East and West, respectively; the letters *u* and *d* denote "up" and "down," respectively, describing the position of the grating. The dashes (—) separate the groups. Thus for the second group of the second plate the first exposure was made with orientation West and grating "up," while the second exposure was made with orientation South and grating "down." The exposures on any one plate were made in rapid succession, the eight exposures, and attendant manipulations, consuming about three minutes. In each group the mid-times of exposures differed by less than thirty sec-

onds, so that the effects of any changes of temperature in the prisms and spectrograph framework were made small.

Data describing the plates and the results of the least squares reductions are shown in the table. Column I contains the plate number, column II the mean of the exposure dates, column III the series number of the plate, columns IV, V, VI and VII the z's (in units of $^1/_{24,000,000}$ inch) of the four groups.

I	II	III	IV	V	VI	VII	VIII	IX
PLATE NO.	DATE U.T., 1935 JULY	SERIES	z_1‡	z_2	z_3	z_4	$v_{1,2}$‡	$v_{3,4}$
E 15468	$8^d\ 20^h\ 35^m$	1	−163	+58	+28	+493	+221	+465
69	8 20 42	2	+193	+107	+166	+ 14	−86	−152
70	8 20 53	3	− 26	+214	− 23	−219	−240	+196
71	9 0 13	1	− 8	−198	+ 10	− 18	−190	− 28
72	9 0 18	2	+ 81	−161	− 80	+ 49	−242	+129
73	9 0 23	3	+ 23	+208*	− 64	−265	−185†	+201
74	9 4 12	1	− 76	+114	+248	+306	+190	+ 58
76	9 4 22	3	+110	+304	−115	−236	−194	+121
77	9 4 27	2	+ 51	−134*	−154	+386	−185†	+540
78	9 16 52	1	−301	+ 92	−284	+456	+393	+740
79	9 16 56	2	+183	+161	+431	0	− 22	−431
80	9 17 1	3	+200	+170	+ 37	+160	+ 30	−123
81	12 8 5	1	− 12	−175	+199	+523	−163	+324
82	12 8 10	2	+ 91	+104	−130	− 32	+ 13	+ 98
83	12 8 14	3	− 19	−167	−171*	−133	+148	− 38†
84	13 12 46	1	+ 91	+120	−122	+515	+ 29	+637
85	13 12 51	2	+328	− 54	− 73	+133	−382	+206
86	13 12 55	3	+259	+275	−178	−211	− 16	+ 33

* An average. Twice usual weight
† Weight $^4/_3$ of usual weight
‡ Unit of z and v: $^1/_{24,000,000}$ inch

The differences $(z_1 - z_2)$ and $(z_3 - z_4)$ contain any displacements due to the different orientations of the members of the groups, but are free from the effects of *uniformly* changing temperature during the exposures of groups 1 and 2, and 3 and 4. The corresponding sums would be free of effects of orientation, but would contain the effects of changing temperature. Analysis of the sums indicates an average rise in temperature of the order of 0°.1C. between exposures in a group. The quantities $v_{1,2}$ and $v_{3,4}$ in columns VIII and IX are defined as follows:

Series	$v_{1,2}$	$v_{3,4}$
1 and 2	$z_2 - z_1$	$z_4 - z_3$
3	$z_1 - z_2$	$z_3 - z_4$

In the units of the table, the meanings of the v's are:

$$\text{Series 1 and 3} \quad \begin{cases} v_{1,2} = (\eta_N - \eta_S) \times 1.17 \times 10^9 \\ v_{3,4} = (\eta_E - \eta_W) \times 1.17 \times 10^9 \end{cases}$$

$$\text{Series 2} \qquad \begin{cases} v_{1,2} = (\eta_W - \eta_S) \times 1.17 \times 10^9 \\ v_{3,4} = (\eta_N - \eta_E) \times 1.17 \times 10^9 \end{cases}$$

where η_i is the index of refraction in the orientation i.

Each least squares solution for z yielded likewise the probable error of z so determined. The probable error of z varied from group to group, in nearly the manner expected for an estimate of a probable error obtained from twelve equations in four unknowns.[2] There was no good evidence for any real variation in accuracy of measurement from group to group, or measurer to measurer. The probable error, obtained from all of the measures, of a single z of unit weight, was 146 in units of $^1/_{24,000,000}$ inch, or, in terms of refractive index, 1.24×10^{-7}. It has already been mentioned, however, that the z's contain small errors, due to irregularities in the spectrograph slit, over and above those linear and quadratic errors eliminated by the least squares solutions. The effects of these small irregularities are two-fold. They introduce a constant error in all of the z's, and they also contribute to the total standard deviation of the least squares residuals and would thus cause, unless allowed for, an overestimation of the probable error of a $v/2$ of unit weight. The v's are free from all effects of slit irregularities. The least squares residuals may be freed from slit irregularities by subtracting from each residual the mean (over all groups) of residuals of the same segment. It is then found that the probable error, arising from errors of measurement, of a single z of unit weight is 128, or 1.088×10^{-7} in the refractive index. The expected probable error of a $v/2$ of unit weight should be $2^{1/2}/2$ times this, or 7.69×10^{-8} in the index of refraction.

On the assumption that the refractive index is really constant within groups, one can compute the "observed" probable error of a single $v/2$ of unit weight from the values listed in columns VIII and IX of the table, for the $v/2$'s can then differ from zero only by errors of measurement. An excess of the "observed" over the expected probable error of a $v/2$ of unit weight would be evidence for the falsity of the assumption, and would thus indicate real changes of refractive index within groups. The observed probable error of a single $v/2$ of unit weight is 7.83×10^{-8} in the refractive index, in good agreement[3] with the expected value. The refractive index is thus found, to an accuracy of about 5×10^{-8}, to be independent of orientation.[4]

Studies were made graphically and analytically to ascertain any correlations of the v's or important combinations of them with the sidereal time. No significant correlations were apparent. A somewhat similar investigation with visual apparatus was carried out by Maxwell[5] who obtained negative results, but the degree of precision in his experiment is not known.

[1] Building A, Cambridge Station, immediately over the pendulum pit.

[2] Sterne, *Proc. Nat. Acad. Sci.*, **20**, 565, equation (8) (1934).

[3] The observed probable error is computed from 36 degrees of freedom, the expected from about 600. The probability of a greater excess of the observed over the expected probable error is about 0.42 from the theory of sampling, and thus the observed excess of 7.83×10^{-8} over 7.69×10^{-8} has no significance.

[4] The weighted mean of the observed $v/2$'s is $2.2 \times 10^{-8} \pm 1.3 \times 10^{-8}$ (p.e.) in the refractive index. In comparison with its probable error, this mean does not differ significantly from zero. It will be seen that an extensive use was made of the method of least squares, which is rigorously correct only when the errors obey the normal law. There were, in all, 900 residuals in the least squares solutions, and these were found to conform to the normal law of errors, with no significant excess of large residuals. The tests were carried out by Pearson's X^2 method. The residuals were also tested for normality by the use of third and fourth moments.

[5] *Phil. Trans.*, **158**, 532 (1868).

IRRADIATION OF PLANT VIRUSES AND OF MICROÖRGANISMS WITH MONOCHROMATIC LIGHT. III. RESISTANCE OF THE VIRUS OF TYPICAL TOBACCO MOSAIC AND ESCHERICHIA COLI TO RADIATION FROM λ 3000 TO λ 2250 Å

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We have described in previous publications[1,2] the effect of monochromatic ultra-violet radiation down to λ 2650 Å (and 2537 Å) on a semipurified suspension of typical tobacco mosaic virus and a number of bacteria in the vegetative and spore stages, irradiated in the same suspension. It has since been possible to purify the virus suspension to a very much higher degree, producing a colorless and clear suspension, highly infective and with relatively little absorption at wave-lengths even as short as 2650 Å to 2250 Å in the dilution used. This high degree of purification has been attained by four simple procedures: (*a*) extraction of a crude extract from diseased plants by grinding and subsequently pressing out through cheesecloth, (*b*) subjecting the extract to a temperature of 65°C. for 15 min., (c) treatment (by stirring) with charcoal (Nuchar *W*) for 1 min., (*d*) filtration and refiltration with suction through hard filter paper.* The material was diluted to 5/100 with physiological salt solution for irradiation.

The bacterial work described in this paper has been conducted with a 15-hour agar slant culture of *Es. coli*. Previously the organism was grown in bouillon, as described in earlier publications, but it was found that even in extremely low concentrations bouillon absorbed highly wave-lengths below 2650 Å. In the present work the exposed suspension contained about 100,000 bacteria per cc. The details of the procedure of preparing

the bacterial suspension, as well as some minor changes in the apparatus, in the method of exposure and in the determination of the effect on the bacteria are described elsewhere.[4]

The infectivity of the virus suspension was determined by a modification of the primary local lesion method first introduced by Holmes[5] and later

TABLE 1

Exp. V_H. Nov. 1, 1934: Typical virus suspension, Nuchar (charcoal) treated; material clear and colorless, diluted 5/100 with physiological salt solution; exposure cell contents, 5 cc.; wave-length, λ 2650 Å; thermopile 11.2 erg/cm. of galvanometer deflection; operating temperature, 18°C.; inoculation of *Nicotiana glutinosa* immediately after irradiation, each run on 20 plants, one leaf per run on each plant; readings taken Nov. 7; all lesions counted.

TIME OF EXPOSURE, MIN.	ERG/CC. INCIDENT ENERGY	TOTAL NO. LESIONS IN 20 LEAVES	AV. NO. LESIONS PER LEAF	SURVIVAL RATIO
Control (3 sets)	0	658 (av. of 3 sets)	31.93	100
10	56,500	609	30.4	92.2
20	113,000	487	24.3	73.7
40	230,000	268	14.5	40.6
80	445,000	203	10.15	30.8
160	890,000	3	0.15	0.5

TABLE 2

All energy values are for 50% inactivation of 1 cc. of standard purified virus (typical tobacco mosaic) and *Es. coli* suspensions. Data are from complete inactivation curves. **E** 2250/**E** (virus) = Energy needed to inactivate at λ 2250 Å divided by energy needed to inactivate at the respective wave-lengths given in column 1 of table; for the bacteria (*Es. coli*), the reference wave-length is λ 2650 Å.

1 WAVE-LENGTH, Å	2 E (VIRUS), ERGS	3 E (*Es. coli*), ERGS	4 E(VIRUS LESS *Es. coli*), ERGS	5 $\frac{E\ 2250}{E\ (VIRUS)}$	6 $\frac{E\ 2650}{E\ (Es.\ coli)}$
2250	27,700	8,500	19,200	1.000	0.498
2300	31,800	9,850	21,950	0.714	0.420
2400	58,000	9,400	48,600	0.477	0.442
2480	133,760	8,100	125,660	0.235	0.514
2537	117,000	6,500	110,500	0.237	0.638
2650	158,000	4,150	153,850	0.175	1.000
2805	300,000	7,000	293,000	0.092	0.693
2950–3050	500,000	13,000	487,000	0.056	0.320

statistically studied by Youden and Beale.[8] *Nicotiana glutinosa* was used as the test plant. The essential modification in the method consisted in using a measured area of each leaf selected and exercising extreme care to employ uniformity in rubbing the inoculum over this area. Each "run"—defined as one exposure of the suspension at any particular wave-length—was tested by inoculation of the exposed or control suspension on 16 to 20

leaves, each leaf on a separate plant, and also arranging that the selected leaves occupied all possible positions on the plants. For other runs, using the same suspension, other leaves on the same plant were utilized. On the average there were about 16 lesions per area with the control suspension at

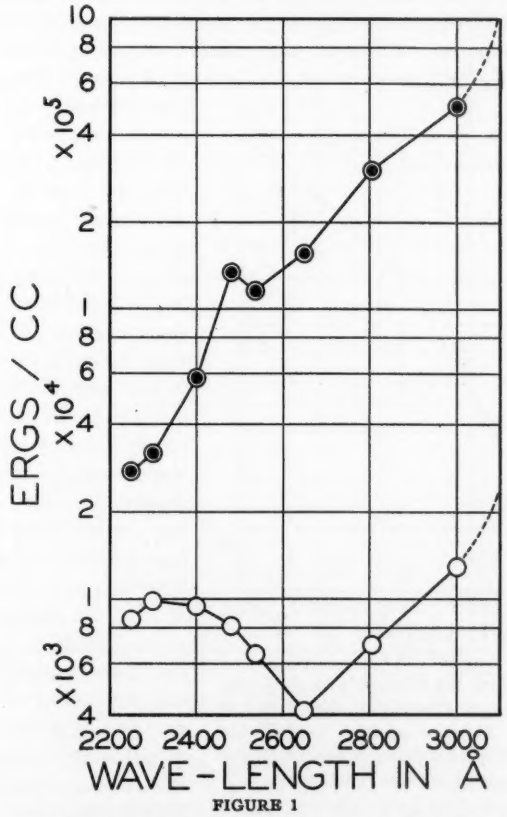

FIGURE 1

Incident energies in erg/cc. necessary to inactivate 50% of standard purified virus (●) and *Es. coli* (○) in the same suspension plotted against wave-length.

the virus dilution employed. Accordingly, the total "survival value" of the control for comparison was about 250 to 320 lesions per run. Altogether about 235 experimental runs were made in addition to about 30 preliminary tests. The data given in table 2 were obtained from more than 70,000 lesions. About 5 to 7 runs per wave-length were made and an inactivation curve was drawn for each wave-length. A typical protocol is

given in table 1. In table 2 there are given values for 50% inactivation of virus and bacteria. The data for the virus are averages of four complete (all wave-lengths) series or sets of data; i.e., the sensitivity for all the wave-lengths was obtained from one batch of material for each series. This was necessary since the absolute energy needed to inactivate the virus suspension varied with the previous history of the plants from which the diseased juice was prepared. However, the relative wave-length dependence as expressed in figure 3 was uniform for each single series. The points in the inactivation curve (Fig. 1), which we consider of special importance, that is, those around λ 2300 Å and 2650 Å were each separately investigated by four experimental series. It was found that 50% of the virus in 1 cc. at 2650 Å was inactivated by 158,000 ergs, whereas at 2250 Å, by 27,500 ergs. This order of magnitude in the sharp decrease toward the shorter wave-lengths, respecting the energy necessary to produce the inactivation effect, has been found in each case. The inflection of the curve around λ 2537 Å shifted sometimes toward 2650 Å, depending on the previous history of the material. The average of our energy values place it at 2537 Å, although it is quite possible that the maximum lies between λ 2537 and 2650 Å.

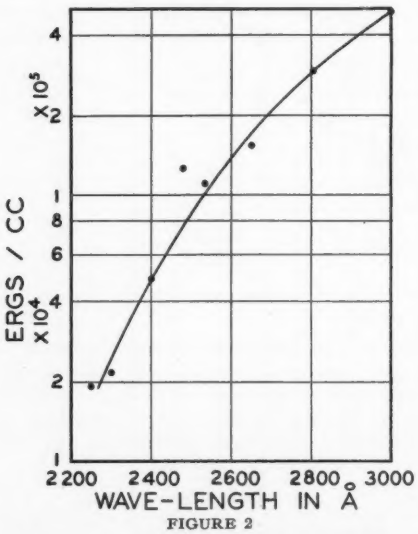

FIGURE 2

E (energy) virus less E bacteria for 50% inactivation in the same suspension plotted against wave-length. At wave-lengths longer than 3100 Å neither virus nor bacteria was inactivated by the energies used in these experiments.

In these experiments the virus has been found to be 40 times more resistant to λ 2650 Å than the bacteria, when irradiated in the same suspension (see table 2, column 4). This is, however, a significantly lesser value than the one given[2] for a less purified virus suspension. The difference is possibly explained by the assumption that the higher purification has liberated the virus which, in the case of the less purified virus suspension, may have been either clumped or attached to large particles.

Bearing in mind the relative resistance of this virus and of this species of bacteria to λ 2650 Å it is very striking and probably highly important that

the energy necessary to inactivate 50% of the virus at λ 2250 Å is not more than 3.1 times the energy necessary to inactivate 50% of the bacteria. This is brought out strikingly in figure 2. At wave-lengths longer than 3000 Å neither the virus nor this species of bacteria was inactivated by energies used in these experiments. In figure 3 there is presented the rela-

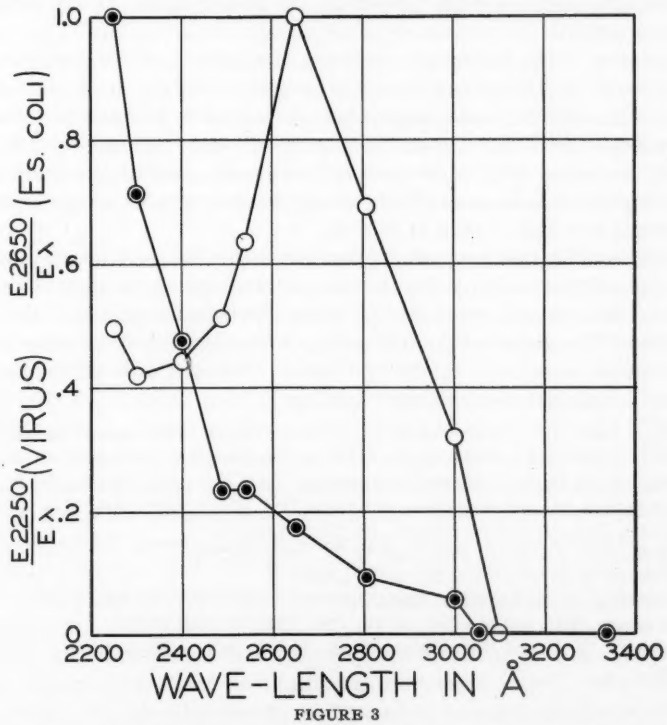

FIGURE 3

The curves show inactivation spectra for virus and bacteria, calculated for virus (●) with reference to **E** (energy) at λ 2250 Å for 50% inactivation, and for bacteria (○) with reference to **E** at λ 2650 Å for 50% inactivation. These curves are given only to show the difference in the location of the maxima. For absolute energies of inactivation figure 1 should be consulted.

tive absorption curve which should have the shape of the reciprocal of the inactivation curve, that is, if all the absorbed energy is used in the inactivation of the material. That the real absorption curve fits the form of the reciprocal of this inactivation curve (Fig. 1) is probable because the virus suspension in the dilution used has relatively little absorption and does not change in absorption measurably during irradiation. (See Warburg.[7])

Attention should be called to the resemblance of the wave-length de-

pendence of the inactivation curves of the virus as described in this paper to that of urease (Kubowitz and Haas[6]) and of pepsin (Gates[3]). Conclusions are, however, premature as long as we have no information (*a*) respecting the wave-length dependence of inactivation of proteins in general at the shorter wave-lengths, as well as (*b*) more information regarding the destruction spectrum of the virus of typical tobacco mosaic at wave-lengths below λ 2250 Å.

Summary.—The destruction spectrum of a highly purified suspension of the virus of typical tobacco mosaic as compared with the destruction spectrum of *Es. coli* in the same suspension is described for λ 2250 Å to λ 3000 Å. It has been found that: (*a*) at λ 2250 Å the amount of energy which is necessary to destroy 50% of the virus in 1 cc. is only one-fifth the amount required at 2650 Å, whereas (*b*) the energy necessary to inactivate bacteria is greater at λ 2250 Å than at 2650 Å.

This investigation has been carried out under the joint auspices (*a*) of the Committee on Radiation, Division of Biology and Agriculture, NATIONAL RESEARCH COUNCIL and (*b*) of the Graduate Committee of the University of Wisconsin and the Wisconsin Alumni Research Foundation. The inoculations were made chiefly by Dean F. McAlister and Tildon Easley, research assistants in virus investigations.

* Since this work was completed Stanley (*Science*, **81**, 1935) has reported the crystallization of the virus of typical tobacco mosaic, and he characterizes the virus as a protein.

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[2] Duggar, B. M., and Hollaender, Alexander, *Ibid.*, **27**, 241–256 (1934).

[3] Gates, F. L., *Jour. Gen. Physiol.*, **18**, 265–278 (1934).

[4] Hollaender, Alexander, and Claus, W. F., *Ibid.* (in press).

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CALCIUM AS A FACTOR IN THE NUTRITIONAL IMPROVEMENT OF HEALTH

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Investigations extending through successive generations of laboratory animals, with natural foods as the experimental variables, have shown[1-3] that a food supply which constitutes a permanently adequate dietary may still be capable of improvement by more scientific adjustment of the quantitative proportions in which the articles of food are consumed.

In these experiments, in which growth was well within the boundaries of the normal, the same nutritional improvement of an already adequate diet has induced both a more rapid growth with somewhat earlier maturity and higher adult vitality with longer life. The growth rates involved have been found[4] to have frequency distributions so symmetrical as to justify a high degree of confidence in the statistical interpretation; and simple statistical treatment of the data indicates clearly that the differences found are unquestionably significant.

Our current experiments seek to analyze these findings in terms of the individual chemical factors involved, beginning with calcium. The minimal level of calcium intake for permanent support of normal nutrition appears, from the findings of several investigations, to lie between 0.13 and 0.19 per cent of calcium in the dry food; while the optimal level is evidently higher.

Both by calcium-balance experiments with growing children[5] and by analyses of the bodies of experimental animals[6,7] it is found that even when there is every appearance of normal growth there may be considerable differences in the rate of calcium retention, depending upon the calcium content of the food.

If such observations stood alone, there might remain a question as to the ultimate effect of such a difference in the rate at which the body develops to its normal adult composition. In the experiments just now completed, which cover the entire life cycle and extend into successive generations, it is found that the diets with moderately increased calcium content, which have expedited growth and development, with earlier attainment of an adult percentage of body-calcium, have also induced higher vitality throughout, and improved the life-expectation of the adult as well as of the young.

With all other factors held uniform,[8] this moderate increase in the calcium content of the food has resulted in more rapid growth, more efficient utilization of food value whether computed in terms of calories or of protein, slightly earlier maturity, improvement of the already normal health at all ages, and some increase in the average length of adult life. In this particular series, the gain in longevity by the males is undoubtedly significant, while that by the females is smaller and if it stood alone would not be statistically convincing. Before one concludes, however, that the nutritional reactions of the sexes are different in this respect, there should be similar experiments comparing the effects of the two food supplies upon the life histories of unmated females, and upon groups of families in which the factor of pregnancy and lactation is held constant. For in the experiments just mentioned, the females on the food supply richer in calcium produced and suckled more young and it is conceivable that if the demands of pregnancy and lactation had been less frequent and heavy these females might

have lived somewhat longer. It is to be hoped that opportunity can be found for such an additional series of experiments.

Meanwhile, experiments are already in progress to determine the effects of further enrichment of the diet in its calcium and phosphorus contents,[9] and with and without simultaneous enrichment in protein content, vitamin values, or both.[10] Inasmuch as other work in this laboratory indicates that both in the case of vitamin A[11] and of vitamin G[12] there is progressive nutritional benefit with increasing liberality of intake up to levels probably at least fourfold higher than the actual or minimal needs of normal nutrition, it seems important to determine, as accurately as possible, the extent of the differences between the minimal calcium requirements of normal nutrition and the intake which induces the best results as revealed by observations extending throughout the life cycle and into successive generations.

With an adequate diet having a calcium: phosphorus ratio of 1.4:1, the level of calcium intake most favorable to general growth is found at 0.64 per cent of the dry food mixture (as suggested by McCollum and Simmonds several years ago); and this high level also increases the rate at which the growing individual approaches the normal adult in its status as to percentage of body calcium. This latter phase of development is expedited still further at the still higher level of intake of 0.8 per cent calcium in the dry food, though here the general growth is slightly less rapid than with food containing 0.64 per cent of calcium. It is planned to continue the studies of the effects of these relatively high levels of intake throughout the life-cycle of at least two generations, and also with a basal diet made up in closer imitation of the average American family food supply.

In so far as can be judged from the considerable amount of evidence now in hand, it would appear that the optimal level of calcium intake is about three times as high as the minimal adequate level which meets the directly demonstrable requirements of normal nutrition; or, in other words, that for best results about three times as much is needed as is required to make the dietary "adequate" in the usual sense of the term.

The coöperation of the Carnegie Corporation of New York and the Carnegie Institution of Washington is gratefully acknowledged.

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[2] H. C. Sherman and H. L. Campbell, *Proc. Nat. Acad. Sci.*, **14**, 852–855 (1928).
[3] H. C. Sherman and H. L. Campbell, *Jour. Nutrition*, **2**, 415–417 (1930).
[4] H. C. Sherman and H. L. Campbell, *Proc. Nat. Acad. Sci.*, **20**, 413–416 (1934).
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VARIEGATION IN DROSOPHILA AND THE INERT CHROMOSOME REGIONS

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At the time of their discovery, the variegated races of Drosophila (D. virilis, Demerec ('26); D. melanogaster, Muller ('30)) seemed to be promising material for the study of mutation. They have since developed rather into a puzzle *sui generis*. Members of the group have been discussed variously as mutable genes (Demerec ('28)), unstable translocations (Muller ('30), Patterson and Painter ('31, '32)) and, more recently, as a rather special type of duplication undergoing frequent somatic crossing-over (Stern ('35)). The discussions have not been very satisfactory; partly because the data themselves have been inadequate for the formulation of general rules, excepting only Muller's correlation of the "eversporting" types in D. melanogaster with the occurrence of chromosome rearrangement.

This paper summarizes the results of experiments with thirteen different variegations in Drosophila melanogaster. All of these belong to Muller's "eversporting displacements"—they are associated with chromosome rearrangements. Five involve the region around the white locus in chromosome 1; two the yellow region of the same chromosome; and the six remaining, the brown region of the second chromosome. I have attempted to determine what relation there might be between the nature of the rearrangements and the production of variegation. To this end, I have studied the characteristics of the rearrangements cytologically, in the salivary gland chromosomes; and the characteristics of the variegations, genetically, in relation to the different affected genes. Both series of data show a relation between variegation and the so-called "inert" regions. The first evidence of the sort came from the work of Gowen and Gay ('34), on the suppression of white-variegation by a supernumerary *Y* chromosome. This may now be extended; extent of variegation, that is, the proportion of "mutant" to wild type tissue, depends upon a quantitative relation between active and "inert" chromosome regions. In addition, however, the "inert" regions are involved in the rearrangements themselves. Thus both the chromosome structure associated with variegation and the extent of variegation are related to the "inert" chromosome regions.

The variegated races form abnormal configurations of the salivary gland chromosomes as a direct result of their relation to the "inert" regions. In a nucleus with normal chromosomes, the "inert" regions are aggregated to

form a "chromocenter" (Heitz ('33, a, b,); Painter ('35)). Since in Drosophila melanogaster these regions are normally located near the spindle attachments of the chromosomes (Heitz ('33), Kaufman ('34)), a configuration is formed in which all spindle attachments are approximated. In the chromosome rearrangements where "inert" regions are transferred from the spindle attachment to other loci, the coalescence to a chromocenter persists in the salivary gland nucleus. The result is that, in nuclei of such types, rings, lateral attachments and more complex configurations may occur.

Ring configurations are regularly found when an "active" chromosome region is intercalated into an "inert" region. For example, both ends of the normal fourth chromosomes of Drosophila melanogaster are regularly part of the chromocenter (Bridges ('35)). Three of the variegations involving the white locus turn out to be intercalations, into the fourth chromosome, of the section of the *X* from yellow to white, or in one case, from yellow to echinus. These form, then, part of a ring configuration. A similar situation exists for the yellow-silver variegation of Sturtevant ('34), scute 10-2. Another variegated white forms a ring as the result of intercalation of a small portion of the *X* chromosome, including white, into the inert region of the left limb of chromosome 3.

Effective "lateral" attachments occur when "inert" region is intercalated between two "active" regions. After chromocenter formation, the "active" region distal to the inert material is attached to the chromocenter by its proximal end; the other "active" region, between the inert material and the spindle attachment, is bent into a loop. Figures of this kind are found in the scute-8 inversion, in two of the variegated brown allelomorphs, and in one of the white variegations. It is readily seen that the principle is the same as in the cases of ring formation. Still more complex configurations are found in other cases, the four remaining variegated brown allelomorphs. They also result from the breakage and rearrangement of inert regions, which by their aggregation to form a chromocenter may then join the broken chromosomes. An apparently similar case has been described by Dubinin and his collaborators ('35), and interpreted as a fusion of specific intergenic bonds. Evidently on the basis of the present discussion this assumption is not yet necessary. Their "reconstruction of a normal chromosome" presumably followed disruption of the chromocenter aggregate when the preparation was smeared.

In addition to the thirteen cases I have studied, those already described by other workers lend themselves to a similar interpretation (see Patterson ('32, '33), Gowen and Gay ('34), Glass ('34a, b), Van Atta ('32), Painter ('34), Mackensen ('35), Patterson and Stone ('35), Stone and Thomas ('35)). There are some variegations, that I am studying, which may be of a different type; and I have seen similar abnormal configurations unac-

companied by obvious variegation. These exceptions are, however, in a minority. The evidence seems adequate to establish the rule, that variegations are associated with abnormal configurations of the salivary gland

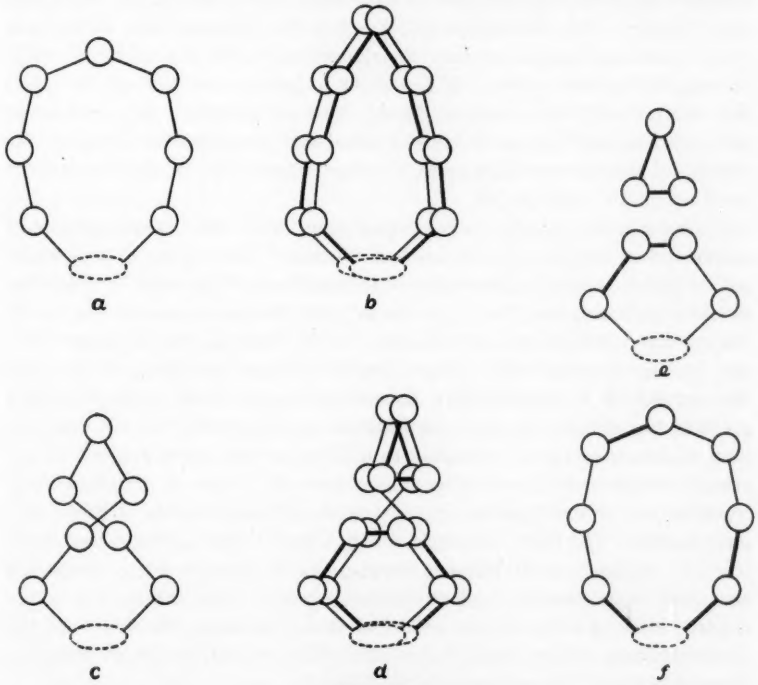

FIGURE 1

Reproduction in normal and twisted ring configurations. The circles represent chromomeres (genes), the ellipses the spindle attachment. Light lines are the old connecting fibres, heavy lines the new ones.

(*a*, *b*) Reproduction in a normal ring; (*c*) a twisted ring; (*d*) reproduction in a ring where distances are such as to permit abnormal linkages to form; (*e*, *f*) the products of reproduction: normal ring, deficient ring and fragment without spindle attachment.

By variation of the type of overlap or the plane of formation of the new fibres, rearrangements, duplications, double or interlocked rings may result.

chromosomes, caused in these cases by the aggregation of inert regions to a chromocenter.

The variegation in all of the rearrangements concerns those genes close to the locus of the break. All the available mutants located in the regions showing abnormal configurations were tested for the display of variegation. The evidence from these tests shows clearly that only those characters

whose genes lie close to the locus of rearrangement as determined in the salivary gland chromosomes are affected. No unequivocal instance has yet been found of the total loss of a fragment as the cause of variegation. One such case has been reported to have been seen cytologically (Patterson and Painter ('31), Patterson ('32)). But the genetic data (Patterson ('32)) indicate variegation only for the white-Notch loci, although tests were made for other genes. If the whole fragment were lost, all the genes that are included in the region should show variegation. It would seem, then, that in this case, as in those studied and recognized as being of this nature by Gowen and Gay, and by myself, only a part of the fragment is involved in the variegation.

Changes in the extent of variegation occur when the normal balance of active and inert regions in the nucleus is upset. The suppression of variegation by an extra *Y* chromosome is not confined to the white-Notch cases studied by Gowen and Gay. A similar phenomenon occurs for the brown variegations (Schultz and Dobzhansky ('34), Dubinin and Heptner ('34)) and for the others as well. I have now determined the effects of changing the number of *Y* chromosomes, on variegation for most of the affected genes in the different stocks. In addition, I have found that not only the inert region from the *Y*, but also its relation to the active regions, is important. The evidence for this comes from the study of many different combinations of variegations, in types with different balance of active and inert region. The different fragments of *X* and *Y* chromosomes have been studied; triploids with different numbers of *Y* chromosomes; intersexes also, and superfemales; and particularly certain duplications for active regions, some of which annul, others of which simulate, the effects of the *Y* chromosome. Here I will discuss the effects on variegation of changing the number of *Y* chromosomes in the diploid.

The variegated brown allelomorph Plum-2 may be taken as an example, since it illustrates most of the relations found also in other cases. Flies containing a Plum-2 second chromosome display, when appropriately tested, four distinct types of variegation: a dominant brown (eye color), a recessive brown (eye color and testis sheath), a "minus" bristle and "light" (eye color). Of these, the first three named lie close to one side of the breakage point; the fourth ("light") is at the other of the two breaks, within the inert region of 2*L*. It will be recalled here, that in Plum-2 homozygotes, all the broken portions of the chromosome are joined at the chromocenter.

The characteristics of these variegations have been studied in all types from the *XO* male to the *XXYY* female. Minus, which is to the right of brown, shows an extent of variegation in the *XO* male comparable to that shown by brown in the *XY* male. The susceptibility to variegation decreases as the number of *Y* chromosomes increases. In the case of the

"light" variegation, an apparently converse relation holds. With increase in the number of *Y* chromosomes, the extent of "light" areas in the eye increases; in the *XO* male, however, the eye is completely wild-type as regards the "light" character. This is to be interpreted as meaning that the product of variegation in this case is wild type, and the original form "light." Support for such an interpretation comes from the study of several other cases involving the "light" locus, in which a similar break in 2*L* has produced a similar "mutation" (position effect) to "light." Thus the variegation in this case is a "reversion"to the wild type. On this basis, the effect of addition of *Y* chromosomes in all cases is to decrease the frequency of variegation.

All the variegations—those for yellow and achaete, for white, split and Notch, and the others for loci in the brown region—exhibit similar characteristics. They cannot be discussed here due to limitations of space. In all cases, however, it appears that the extent of variegation increases as the *Y* chromosome number is lowered. The result is that the apparent direction of "mutation," as judged by relative proportions of mutant and wild type tissue, may appear to change with change in *Y* chromosome number. These relations suggest that the origin of variegation may be considered as the result of some type of unstable configuration which produces different types of "mutation" in the different *Y* chromosome types. The presence of the abnormal chromosome configurations in the salivary gland nuclei permits an attempt to formulate an hypothesis based on consideration of the reproduction of genes in these configurations.

The basic postulate for a theory of the variegations involves the nature of the so-called "mutations." Either they are intra-genic, the orthodox mutation types; or they involve structural changes of the chromosomes. In one of the present cases—scute-8—Beadle and Sturtevant (in the press) have shown that germinal deficiency for the variegating portion of the chromosome may occur. Other data, on different variegation types, may be interpreted similarly. These belong then to the simplest type of structural change—a loss which occurs without a complementary production of duplications. There are, in addition, a number of instances of variegation which are definitely different from known deficiencies for the same loci. Examples are the dominant brown variegations, and certain white variegations. These may be interpreted as due to position effects, effectively mutations in appearance, resulting from local rearrangements produced by a mechanism similar to that which produces the deficiencies.

It has long been obvious that losses or rearrangements of genes might occur as a result of the difficulties of division in abnormally shaped chromosomes (see especially the results of McClintock ('32) on ring-shaped chromosomes in Zea mays). It is possible to consider such a process simply, following Belling's ('33) analysis of gene (chromosome) reproduction into

two components. These are the synthesis of a new gene, and the maintenance of the genes in linear order. According to his view, the mechanism which maintains linear order operates after the genes have reproduced. Thus, whenever gene reproduction occurs in crossed chromatids, the new linear connections are formed between the closest genes—hence, according to Belling, crossing-over. This makes no postulate as to the nature of the new "fibre"; what is discussed is the time of its formation.

The application of such an analysis to the variegations is simple. The reproduction of a ring-shaped chromosome will offer a model for consideration. From a normal ring, two identical daughters result (Figs. 1*a*, *b*). But if the ring is twisted so that two non-homologous portions come to lie one over the other, the reproduction is abnormal. When the new fibres form across the shortest way (Fig. 1*d*), a normal ring, a small ring and a fragment lacking a spindle attachment, result under the conditions shown in the figure. Evidently the formation of rings of different sizes, observed by McClintock in Zea mays, might be explained in this way. Moreover, only rings would ordinarily be produced from rings. In addition, by variations of details in the place of twist, or the plane in which the new intergenic connections are formed, a variety of other new rings are possible. These may be gene rearrangements within the ring, or duplications which would appear as larger rings. Further discussion, and the detailed application of the hypothesis to the various cases, is deferred until the full presentation of the data.

The effect of the *Y* chromosome and other inert regions on the extent of variegation may be derived from this point of view, with an additional consideration. I have found, in comparative studies of larvae having different numbers of *Y* chromosomes, differences in the "turgor" of the salivary gland chromosomes. With the increase in number of *Y* chromosomes, all the others show an increase both in the sharpness of stain and the plumpness of the chromosomes. This result may possibly be related to the development of accessory chromosome materials, in a manner similar to the suggestion of McClintock that the nucleolar center in chromosome 6 of Zea mays, has to do with the development of chromosome matrix. Here also a nucleolar center—that of the *Y* chromosome—may be concerned. It is clear that any factor changing the "turgor" of the chromosomes would have an influence on the frequency of cross contacts of non-homologous genes. This would appear as a change of the sort found in the extent of variegation.

The hypothesis that I have indicated for variegation is to be regarded as a pictorial summary of the facts, cytological and genetic, about the variegations. A variety of other hypotheses are possible; differences in rate of gene division, or of formation of intergenic connections; or a whole variety of intragenic changes may be invoked. None that I have considered are

so simply related to the general body of cytogenetic theory as the one I have discussed, although even it has its difficulties. It is perhaps worth noting, that in its more general aspects, the theory presents the study of variegations as a study of the mechanism whereby the genes are maintained in linear order.

[1] A bibliography will be given with the detailed publication of the data.

[2] The terminology is obviously outworn: "inert" is certainly a misnomer. Heitz's use of "heterochromatin" and "euchromatin" for "inert" and active regions is probably better. For the present, however, I have used the older term because of its familiarity.

SPECIAL REGIONS OF REGULARITY OF FUNCTIONS OF SEVERAL COMPLEX VARIABLES

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Introduction.—In the theory of functions of several complex variables various types of regions occur as convergence regions of series developments of analytic functions.[2] We have investigated the problem of the analytic continuation of a function beyond its associated convergence regions. In this note we present a summary of the results of this investigation. A detailed treatment of the subject will be given in a paper which will appear elsewhere.

Let

$$f(x, y) = \sum_{m, n = 0}^{\infty} a_{mn} x^m y^n \tag{1}$$

be any analytic function of the two complex variables x, y, regular at the origin. We shall discuss the analytic continuation of $f(x, y)$ into three classes of regions.

1. *Diagrams.*—With $f(x, y)$ let us associate the class of *Borel transforms*

$$F(x, y;\ p, q) = \sum_{m, n = 0}^{\infty} \frac{a_{mn} x^m y^n}{(pm + qn)!}, \quad (p, q, \text{positive integers}) \tag{2}$$

Each of these transforms is easily seen to be an entire function. By considering the growth of $F(x, y;\ p, q)$ we define real-valued functions

$$h^*(x, y;\ p, q) = \limsup_{\rho = \infty} \frac{\log \left| F(x\rho^p, y\rho^q;\ p, q) \right|}{\rho}, \tag{3}$$

$$h(x, y;\ p, q) = \limsup_{x' = x,\ y' = y} h^*(x', y';\ p, q), \tag{4}$$

in terms of which we define the (p, q)-diagram D_{pq} of $f(x, y)$.

A point (x, y) belongs to the (p, q)-diagram D_{pq} if and only if there exists a real value θ_0 of θ such that

$$h(xe^{ip\theta_0}, ye^{iq\theta_0};\ p, q) < \cos\theta_0. \tag{5}$$

Pólya's investigations on functions of one complex variable[3] furnish information concerning the region of regularity of $f(xz^p, yz^q)$, considered as a function of z for fixed (x, y). Using this information and applying Hartogs' classical theory,[4] we obtain information concerning $f(xz^p, yz^q)$, as a function of x, y and z, which in turn leads to the following result:

THEOREM 1. *The function $f(x, y)$ is regular at every point of the (p, q)-diagram D_{pq}.*

2. *Borel Regions of Summability.*—Let p, q be any pair of positive integers and α, β any pair of complex numbers such that $|\alpha| + |\beta| \neq 0$. In the z-plane draw a line from the origin to each point z' which is such that the point $(x = \alpha z'^p, y = \beta z'^q)$ is a singular point of $f(x, y)$. Denote that (open) portion of the z-plane bounded by perpendiculars to these lines at the points z' and including the origin by $b_{pq}^{\alpha\beta}$. As the point z runs over $b_{pq}^{\alpha\beta}$ let the point $(x = \alpha z^p, y = \beta z^q)$ run over a point-set $B_{pq}^{\alpha\beta}$. With the same construction for every pair of complex numbers α, β for which $|\alpha| + |\beta| \neq 0$, we obtain a portion of the x-y-space which we call the *Borel (p, q)-region of summability B_{pq} of $f(x, y)$*.

Borel's classical theory furnishes us with information concerning $f(xz^p, yz^q)$, considered as a function of z. Using Hartogs' theory, we obtain the following result:

THEOREM 2. *The series development*

$$f(x, y) = \sum_{\nu = 0}^{\infty} \Big\{ \sum_{pm + qn = \nu} a_{mn} x^m y^n \Big\} \tag{6}$$

of $f(x, y)$ is absolutely summable by Borel's integral means in a neighborhood of every point of B_{pq}. It is not absolutely summable in a neighborhood of any point exterior to B_{pq}.

3. *Star Regions.*—Let σ, μ be any two positive numbers. Let us consider a curve

$$x = \alpha\rho^\sigma, y = \beta\rho^\mu, (0 \leqq \rho < \infty), \tag{7}$$

where α, β are any complex numbers such that $|\alpha| + |\beta| \neq 0$. If the analytic continuation of $f(x, y)$ has no singular point along this curve, we retain the whole curve. If $(x_0 = \alpha\rho_0^\sigma, y_0 = \beta\rho_0^\mu)$ is the first singular

point of $f(x, y)$ along this curve, we retain only the curve up to the first singular point excluding that point. With the same construction for every pair of complex numbers α, β for which $|\alpha| + |\beta| \neq 0$, we obtain a portion of the x-y-space which we call the *(σ, μ)-star region $S_{\sigma\mu}$ of $f(x, y)$*. If $(x_0 = \alpha\rho_0^\sigma, y_0 = \beta\rho_0^\mu)$ is the first singular point of $f(x, y)$ on the curve (7), then we call (x_0, y_0) *the vertex of $S_{\sigma\mu}$ belonging to the curve* (7).

Let a be any positive number and let us define the entire function

$$F_a(x, y;\ \sigma,\ \mu) = \sum_{m,\,n\,=\,0}^{\infty} \frac{a_{mn}x^m y^n}{\Gamma(1 + a\sigma m + a\mu n)} \tag{8}$$

We define two real-valued functions

$$c^*(x, y;\ \sigma,\ \mu) = \lim_{a=0}\lim_{\omega=\infty} \sup \left\{a \log^{+} \log^{+} \left| F(xe^{\sigma\omega}, ye^{\mu\omega};\ \sigma,\ \mu)\right| - \omega\right\}, \tag{9}$$

$$c(x, y;\ \sigma,\ \mu) = \lim_{x'=x,\ y'=y} \sup\, c^*(x', y';\ \sigma,\ \mu), \tag{10}$$

where $\log^{+} A$ denotes $\log A$ if $A \geqq 1$, and 0 if $A < 1$.

Using Riesz's results on Dirichlet series[5] and Hartogs' results, we derive the following result:

THEOREM 3. *The (σ, μ)-star region $S_{\sigma\mu}$ of $f(x, y)$ is identical with the set of points (x, y) for which $c(x, y;\ \sigma, \mu) < 0$ and its vertices are given by the points (x, y) for which $c(x, y;\ \sigma, \mu) = 0$.*

[1] NATIONAL RESEARCH FELLOW.

[2] For a discussion of the theory of functions of several complex variables see, for example, H. Behnke and P. Thullen, *Theorie der Funktionen mehrerer komplexer Veränderlichen, Erg. Math. Grenzgebiete*, Berlin (1934).

[3] G. Pólya, *Math. Zeitschrift*, **29**, 549–640 (1929).

[4] F. Hartogs, *Math. Annalen*, **62**, 1–88 (1906).

[5] M. Riesz, *Acta Math.*, **35**, 253–270 (1912). See also B. Almer, *Ark. Mat. Astron. Fys.*, **17**, 1–70 (1922), where stars of the sort examined here are considered.

DISCONTINUOUS GROUPS AND ALLIED TOPICS. I

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The topological theory of discontinuous groups[1] does not contain the results of the previous theories, not even in the case of transformations without fixed points. In particular it does not give the existence of a fundamental domain as defined by van der Waerden in his report on groups of linear transformations.[2]

In these notes I intend to show that the introduction of a new axiom, intermediary to metrisability and local perfect separability, is sufficient for a more complete theory with applications to the units in hypercomplex systems and to the characterization of plane translations. The first note contains a preliminary discussion of the new axiom; some covering theorems are derived—(2,3), (2,5)—which are the basis for the special results. In the final paper the new axiom will be replaced by a natural generalization such as to get away from the first axiom of countability. I shall discuss then the relations to other theories of covering (Borel, Chittenden, Denjoy, Lindelöf, Menger, Robinson), generalized separability (Haratomi) and development in interval-like systems (Alexandroff-Urysohn, Lefschetz, Menger, Moore). Finally I shall determine to what extent the theory of dimension is preserved and enlarged, particularly with respect to the new concept—[1,5]—of "arbitrarily small."

A Hausdorff-space S may be given by a system $\mathfrak{U}$ of neighborhoods U. Without loss of generality we suppose that each U is a neighborhood of all its points.

[1,1] Any system of open sets equivalent to $\mathfrak{U}$ is a "defining system."

[1,2] A point p is limit point of a system $\mathfrak{T}$ or sets T, if each neighborhood of p has points in common with an infinity of T's.

[1,3] A system without limit points is called *discrete*.

(1,1) A subsystem of a discrete system is discrete. The sum $\sum\mathfrak{T}_i$ of a finite number of discrete systems is discrete. If to each set T of a discrete system we assign a subset T', the system of all T' forms a discrete system too.

(1,2) The closures (or boundaries) of a discrete system form a discrete system; the closure of the sum $\sum_{T\in\mathfrak{T}} T$ is the sum of the closures; the boundary of the sum is contained in the sum of the boundaries. If the T's are closed (*or* nowhere dense) the sum has the same property.

[1,4] A system $\mathfrak{A}$ is covered by a system $\mathfrak{B}$, if to each A there is at least

one B containing A. A *covering system* (with respect to the space in discussion) is a system of open sets covering the system of all one-point sets.

[1,5] Suppose that in a certain class of systems we can always find an individual system covered by an assigned covering system. Then we say that this class contains arbitrarily small systems.

(1,3) E.g., there exist arbitrarily small defining systems. For the subsystem of any defining system, which is covered by a given covering system is defining.

Now let $\mathfrak{D}$ be a discrete covering system of sets D. Take all points situated on the boundary of at least one D; all these form a nowhere dense set B. Every other point p lies either in the interior or the exterior of any particular D, together with a suitable neighborhood U_D. If we choose a neighborhood of p having points in common with a finite number $D_1 \ldots D_r$ of D's, we may take U' in the crosscut of the U_{D_i} and thus independent of D.

So we may decompose the set S-B (which is open and everywhere dense) in mutually exclusive open sets J, collecting in the same J all points of S-B lying exactly in the same D's.

The system $\{J\}$ is a discrete covering of S-B; it is covered by $\mathfrak{D}$.

(1,4) Every point of S lies either in a J or on the boundary of a J.

[1,6] A subset of S is called a *piece* if it is the closure of an open set.

Then we may say:

(1,5) Each discrete covering system $\mathfrak{D}$ of (open) sets D defines a "decomposition" of S in pieces $\overline{J}$. The crosscuts of different pieces lie on their boundaries. The pieces form a discrete system; each point lies in a finite number of pieces. The system of the pieces is covered by the system of the closures $\overline{D}$.

[1,7] The (finite) set of pieces containing a point p is called the "star" of p (with respect to the decomposition); the interior of the star is an open set containing p.

In the next paragraph we shall introduce a new axiom which guarantees the existence of arbitrarily small decompositions.

[2,1] A defining system $\mathfrak{U}$ is called "*analysing*" (later: ω-analysing), if to each point p and each U containing p there exists a p-neighborhood $V = \varphi(U, p) \subset U$ such that every U' which has points in common with V is a subset of U, a finite number $U_i(U, p)$ excepted.

Then our new axiom will be:

AXIOM: *The space S possesses an analysing system.*

[2,2] A space satisfying Hausdorff's axioms and the one just stipulated is called "*subanalytic.*"

(2,1) An analysing system $\mathfrak{U}$ contains a subsystem, which is a *discrete* covering system of the space.

This system may be chosen explicitly as the system of all U's, which are maximal, i.e., not contained as a proper subset in any other element U' of $\mathfrak{U}$.

Considering [1,3] we obtain the covering theorem:

(2,2) *A subanalytic space possesses arbitrarily small discrete coverings.*

In general the elements D of this covering will contain smaller U's; if this is not the case one proves by means of the 4-th Hausdorff-axiom that they are one-point sets. If we remove from $\mathfrak{U}$ all the D's which are not minimal, we may find a smaller covering $\mathfrak{D}_2$ of the same type. Continuing this one obtains a sequence of coverings $\mathfrak{D}$, $\mathfrak{D}_2$, $\ldots\mathfrak{D}_n\ldots$ with the following property:

(2,3) Given p, U_p and two sequences D_n, D_n^* ($\subset \mathfrak{D}_n$) such that $D_n \ni p$, $D_n . D_n^* \neq 0$.
Then for almost all n both D_n and D_n^* are in the neighborhood U_p.

It is known,[3] that this property of S implies metrisability:

(2,4) A subanalytic space is metrisable.

One may prove that the union of the sequence $\mathfrak{D}_n$ is a defining system, such that S possesses a defining system which is representable as the limit of a sequence of *discrete* subsystems. I propose to denote this by the term "ω-separability."

In various ways one may secure to each covering $\mathfrak{C} = \{C\}$ another one $\{C'\}$, such that the closures $\bar{C}'$ are covered by $\mathfrak{C}$. Combining this with (1,5) and (2,2) we obtain:

(2,5) *A subanalytic space possesses arbitrarily small decompositions in pieces.*

(3,1) A metric space which has arbitrarily small decompositions into pieces is subanalytic.

(3,2) Any subspace of a subanalytic space is subanalytic.

(3,3) The fundamental cube of Hilbert space is subanalytic.

(3,4) *Every perfectly separable metric space is subanalytic.* This is a result of Lefschetz.[4]

(3,5) If S is locally compact, the elements of sufficiently small coverings have points in at most a finite number of others.

* Sterling Fellow.

[1] Baer and Levi, *Math. Zeit.*, **34**, 110 (1931).

[2] *Ergebnisse der Mathematik*, etc., **4**, 2, p. 35.
[3] Cf., e. g., Kuratowski, *Topologie*, p. 106.
[4] "On Separable Spaces," *Ann. Math.*, **33**, 525 (1932).

DISCONTINUOUS GROUPS AND ALLIED TOPICS. II

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In the preceding note[1] I have established for a certain class of spaces the existence of decompositions into pieces $\bar{J}$, such that (α) the open sets J are mutually exclusive, (β) their sum is everywhere dense, (γ) the sum of the pieces $\bar{J} \overset{3}{\longleftarrow} p$, the star of the point p, contains a neighborhood of p, (δ) the pieces form a locally finite system, (ϵ) the pieces and even the stars may be chosen arbitrarily small. It is important that "arbitrarily" refers to any covering of the space S by open sets.

In particular this "*decomposition theorem*" is true for separable metric spaces. The rôle of these and other decompositions will be studied later[2] in connection with generalized separability, generalized metrisability and subanalyticity; in this note we shall be concerned with applications to locally topological mappings and discontinuous groups.

[1,1] A mapping $\mathfrak{p} = \tau(p)$ of a space S on a Hausdorff space $\mathfrak{S}$ is said to be locally topological, if to each p there is a neighborhood U_p such that $U_p \longleftrightarrow \tau(U_p)$ is topological. If in addition the function U_p may be chosen such that $\tau(U_p) = \tau(U_q)$ implies either $U_p = U_q$ or $U_p \cdot U_q = 0$, τ is called *involutorial*.

Supposing that τ is involutorial and that the decomposition theorem holds in $\mathfrak{S}$, we have

(1,1) There exists in S an open set F, the *fundamental domain*, such that $\alpha(\tau$ is topological in F, $\beta)\tau(\bar{F}) = \mathfrak{S}, \gamma)$ if M is a discrete subset of $\bar{F}$, $\tau(M)$ is discrete.

This theorem is only a member of a chain of relations connecting decomposition- and (later) connection-properties of $\mathfrak{S}$ with the behavior of fundamental domains obtained by the following procedure: Cover $\mathfrak{S}$ by open sets $\tau(U_p)$; take a decomposition such that to each piece $\mathfrak{J}$, a containing $\tau(U_p)$ is assigned; to each of these $\mathfrak{J}$ determine one local inversion. Then F shall be the sum of the originals of the (mutually exclusive!) sets $\mathfrak{J}$ in these topological correspondences.

Now let Γ be a group of topological transformations γ of a space S into

itself. We suppose that $\gamma p = p$ implies that γ is identity; later on we shall allow a set of exceptional points which is nowhere dense.

We define $\tau(p) = \mathfrak{p}$ as the set of all points $\gamma(p)$, $\gamma \epsilon \Gamma$. The system $\mathfrak{S}$ of these sets $\mathfrak{p}$ is made a topological space in defining $\tau(U_p)$ as neighborhoods in $\mathfrak{S}$.

[2,1]Γ is called *discontinuous*, if τ is involutorial and if the space $\mathfrak{S}$ has the decomposition property.

If S is a separable metric space one may omit the second condition.

For a fundamental domain (cf. (1,1)) F we obtain

(2,1) To each p in S there is a q in $\overline{F}$ and a γ in Γ such that $p = \gamma(q)$. The system of all $\gamma(\overline{F})$ forms a locally finite decomposition for S.

(2,2) If $\mathfrak{S}$ is compact, $\overline{F}$ has only a finite number of "neighbors" $\gamma(\overline{F})$, $\gamma(\overline{F}) \cdot \overline{F} \neq 0$.

(2,3) If S is connected and $\overline{F}$ has a finite number of neighbors, Γ may be generated by a finite number of elements.

Baer and Levi[3] define a fundamental domain by $\overline{\tau(F)} = \mathfrak{S}$. The following example illustrates the difference: Let S be the real numbers, Γ the group of translations by integral numbers; let F contain the numbers x_n satisfying $\frac{1}{n+2} < \left| x_n - n \right| < \frac{1}{n+1}$ for $n = 1,2\ldots$ etc.—As an application of (2,3) one obtains the existence of finite generating systems for certain groups of units in hypercomplex systems.

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[1] "Discontinuous Groups and Allied Topics. I," These PROCEEDINGS, **22**, 36-39 (1936).

[2] In a paper on subanalytic spaces.

[3] "Stetige Funktionen in topologischen Räumen," *Mathematische Zeitschrift*, Bd. 34.

CONCEPTS OF NEGATIVE DEGREE

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Apart from the negative degrees subsequently to be envisaged, *concepts* are to be understood as comprising propositions, attributes and relations. The *degree* of a concept is 0, 1 or n (>1) according as the concept is a proposition, an attribute or an n-adic relation. A concept of degree m is called *m-adic* (*medadic, monadic, dyadic,* etc.). The notation '$x_1 \ldots x_m \epsilon \alpha$' will be used to mean that the entities x_i exhibit in that order the m-adic concept α; the proposition thus expressed is specifically the proposition α, or the proposition that x_1 has the attribute α, or the proposition

that the x_i exhibit in that order the m-adic relation α, according as m is 0, 1 or more.

Concepts are to be conceived extensionally, in the sense that m-adic concepts α and β are to be identified if, for all entities x_i, $x_1 \ldots x_m \epsilon \alpha$ if and only if $x_1 \ldots x_m \epsilon \beta$. In particular, taking m as 0, it follows that there are just two medadic concepts or propositions, the true and the false; these will be called T and F. More generally, it follows that any m-adic concept is uniquely determinable by specifying what entities exhibit it (and in what order, where $m > 1$). The propositions can thus be viewed as truth-values, the attributes as classes and the relations as relations in extension. The notation '$x_1 \ldots x_m$—' (which, where $m = 0$, is '—') will be used to represent the m-adic concept exhibited by just such entities x_i, in that order, as satisfy the condition '—'.

Concepts of *type 1* comprise propositions, attributes of individuals and relations of individuals, but exclude attributes of concepts and relations of concepts. I.e., where α is an m-adic concept of type 1, significance of '$x_1 \ldots x_m \epsilon \alpha$' requires the x_i to be individuals rather than concepts in turn. Elsewhere[1] I have presented primitives and definitions for a calculus which embraces all concepts of type 1 as elements, and which, like the simple propositional calculus, depends neither upon use of bound variables nor upon any metamathematical classifying of elements. Since individuals are not included among the elements, the forms of notation '$x_1 \ldots x_m \epsilon \alpha$' and '$x_1 \ldots x_m$—' have no place in the calculus; in terms of these extraneous notational devices, however, supplemented by the familiar notation of truth-functions and quantification, we can describe five basic functions of the calculus itself as follows:

$$(\alpha \uparrow \beta) = x_1 \ldots x_{m+n} (x_1 \ldots x_m \epsilon \alpha . x_{m+1} \ldots x_{m+n} \epsilon \beta). \quad (1)$$

$$(\alpha \text{“} \beta) = x_1 \ldots x_{m-n} (\exists x_{m-n+1} \ldots x_m)(x_1 \ldots x_m \epsilon \alpha . x_{m-n+1} \ldots x_m \epsilon \beta). \quad (2)$$

$$(\alpha ,, \beta) = x_{n+1} \ldots x_m (\exists x_1 \ldots x_n)(x_1 \ldots x_m \epsilon \alpha . x_1 \ldots x_n \epsilon \beta). \quad (3)$$

$$-\alpha = x_1 \ldots x_m \sim x_1 \ldots x_m \epsilon \alpha. \quad (4)$$

$$\mathrm{I}\alpha = x_1 \ldots x_m x_1 \ldots x_m x_1 \ldots x_m \epsilon \alpha. \quad (5)$$

$(\alpha ,, \beta)$ is derivable from the rest, viz., as $((\mathrm{I} - (\mathrm{I}\alpha \text{“} - \alpha) \text{“} \beta) \text{“} \alpha)$, and F and T are definable respectively as $(\alpha \text{“} - \alpha)$ and $-\mathrm{F}$. $(\alpha \text{“} \beta)$ and $-\alpha$ are derivable in turn (following D1–D3, D5, D8[1]) from a single binary function $(\alpha * \beta)$, describable as $-(\alpha \text{“} \beta)$. (All these remarks also hold when the signs ',,' and ' “ ' are interchanged.) Just the functions $(\alpha \uparrow \beta)$, $(\alpha * \beta)$ and $\mathrm{I}\alpha$ then remain as primitive.

Suppose *Principia Mathematica* extended to the treatment of the successive degrees above 2, by a continuation of its methods, and consider

all the expressible functions with respect to which type 1 is closed. They exclude, e.g., the functions $x \epsilon \alpha$, xRy, $\iota'x$, $s'\kappa$ and $\overrightarrow{R}'x$, but include the truth-functions of the propositional calculus, the Boolean functions of classes (including the copulas and predicates $\alpha = \beta$, $\alpha \subset \beta$, $\exists\alpha$, etc.), and the analogous Boolean functions of relations of each degree, as well as such further functions of dyadic-relational theory as $\alpha \uparrow \beta$, $R''\beta$, $D'R$, $R \upharpoonright \beta$, $\breve{R}$, and $Q \mid R$, and more complex functions corresponding to all higher degrees. These constitute, roughly, the range of functions definable on the basis of the primitives presented above, except that each of the functions as so redefined is restricted to type 1 for its arguments (these alone being elements of the calculus), and at the same time ceases to be restricted to fixed degrees for its arguments.

(1)–(5) show that $(\alpha \uparrow \beta)$, $-\alpha$ and $I\alpha$ are respectively $(m + n)$-adic, m-adic and $2m$-adic, and that $(\alpha''\beta)$ and $(\alpha,,\beta)$ and consequently also $(\alpha^*\beta)$ are $(m - n)$-adic, where m and n are the respective degrees of α and β. Where $m < n$, (2) and (3) afford no interpretation for $(\alpha''\beta)$ and $(\alpha,,\beta)$, nor, therefore, for $(\alpha^*\beta)$. Still, the primitive functions must admit all elements as arguments without restriction if the calculus is to depend upon no metamathematical classification of elements. This condition is readily fulfilled by providing some arbitrary supplementary interpretation for the cases of $(\alpha^*\beta)$ where $m < n$. Some derivative interpretation will then accrue to the corresponding cases of $(\alpha''\beta)$ and $(\alpha,,\beta)$, through the definitions of the latter functions in terms of $(\alpha^*\beta)$. Whereas $(\alpha^*\beta)$, $(\alpha''\beta)$ and $(\alpha,,\beta)$ are $(m - n)$-adic where $m \geqslant n$, in the cases where $m < n$ these concepts will perhaps be medadic, or $(n - m)$-adic, or $2(n - m)$-adic, or even of unlike degrees, depending upon the supplementary interpretation of $(\alpha^*\beta)$. This interpretation can be so fashioned, incidentally, as to provide for the derivability of $I\alpha$ as a by-product, thus reducing the primitive functions to $(\alpha \uparrow \beta)$ and $(\alpha^*\beta)$.[1]

But, as an alternative to the above procedure of supplementary interpretation, we might follow arithmetical precedent and invent supplementary elements: concepts of negative degree, specifically, by way of countenancing $(\alpha''\beta)$, $(\alpha,,\beta)$ and $(\alpha^*\beta)$ as $(m - n)$-adic concepts even where $m < n$. As with imaginary numbers, no intuitive meaning is contemplated for these concepts of negative degree; their utility is structural simplification. In choosing properties for these new fictitious elements our guide may be the maintenance, over the new range of generality, of various principles which obtained throughout the original realm of natural (non-negative) degrees. It happens that we cannot preserve all such principles over the added range without providing thereby for the deducibility of further principles which are invalid among the natural degrees themselves; but we can preserve many, and have some choice.

Among the principles obtaining throughout the natural degrees there are, e.g., the following identities; study of (1)–(3) reveals them as holding for all those values of the variables for which (2) and (3) yield interpretation at all.

$$((\alpha\uparrow\beta)\uparrow\gamma) = (\alpha\uparrow(\beta\uparrow\gamma)). \tag{6}$$

$$(\alpha\text{``}\alpha) = (\alpha,,\alpha) \quad [= (\exists x_1 \ldots x_m)x_1 \ldots x_m \epsilon\alpha]. \tag{7}$$

$$\begin{aligned}((\alpha\uparrow\alpha)\text{``}\alpha) &= ((\alpha\uparrow\alpha),,\alpha) = ((\alpha\text{``}\alpha)\uparrow\alpha) = (\alpha\uparrow(\alpha\text{``}\alpha)) = \\ &(\alpha\text{``}(\alpha\text{``}\alpha)) = (\alpha,,(\alpha\text{``}\alpha)) = (\mathrm{T}\uparrow\alpha) = (\alpha\uparrow\mathrm{T}) \\ &= (\alpha\text{``}\mathrm{T}) = (\alpha,,\mathrm{T}) = \alpha.\end{aligned} \tag{8}$$

$$((\alpha\text{``}\beta),,\gamma) = ((\alpha,,\gamma)\text{``}\beta). \tag{9}$$

$$((\alpha\text{``}\beta)\text{``}\gamma) = (\alpha\text{``}(\gamma\uparrow\beta)). \quad (10a) \quad ((\alpha,,\beta),,\gamma) = (\alpha,,(\beta\uparrow\gamma)). \quad (10b)$$

$$((\alpha\uparrow\beta)\text{``}\gamma) = (\alpha\text{``}(\gamma\text{``}\beta)). \quad (11a) \quad ((\alpha\uparrow\beta),,\gamma) = (\beta,,(\gamma,,\alpha)). \quad (11b)$$

$$((\alpha\uparrow\beta)\text{``}\gamma) = (\alpha\uparrow(\beta\text{``}\gamma)). \quad (12a) \quad ((\alpha\uparrow\beta),,\gamma) = ((\alpha,,\gamma)\uparrow\beta). \quad (12b)$$

Within the natural degrees only (6)–(8) are significant for all values of the variables. The ranges of significance of (11a) and (12a) barely meet, for (11a) requires that $k \geqslant n$, whereas (12a) requires that $k \leqslant n$, where k and n are the respective degrees of γ and β. Analogously for (11b) and (12b).

Recognition of negative degree releases (9)–(12) from their restricted ranges of significance and renders them significant, like (6)–(8), for all values of their variables. It also increases the scope of all the identities (6)–(12) to the extent of supplying the concepts of negative degree as further values for the variables. We have yet to decide to what extent these identities are to continue true in their increased range of significance. It will be found that retention of the set (6)–(12) over the increased range involves consequences which are invalid among the natural degrees themselves; without (6), on the other hand, the set is not known to involve such consequences.

Let us then surrender (6) in the general case, though of course retaining it for natural degrees, and consider a few of the consequences of retaining (7)–(12) in the general case. From (11a) and (12a), whose ranges of significance now coincide and are universal, there follows the identity of the right side of (11a) with that of (12a); similarly for (11b) and (12b). Thus

$$(\alpha\text{``}(\beta\text{``}\gamma)) = (\alpha\uparrow(\gamma\text{``}\beta)). \quad (13a) \qquad (\alpha,,(\beta,,\gamma)) = ((\gamma,,\beta)\uparrow\alpha). \quad (13b)$$

Further identities proceed as follows. The identity justifying each is indicated by a subscript.

$$(\alpha\text{``}(\alpha\uparrow\alpha)) =_{10a} (\alpha\text{``}\alpha)\text{``}\alpha) =_7 ((\alpha,,\alpha)\text{``}\alpha) =_9 ((\alpha\text{``}\alpha),,\alpha) =_7 ((\alpha,,\alpha),,\alpha) =_{10b} (\alpha,,(\alpha\uparrow\alpha)). \quad (14)$$

$$(\mathrm{T}\text{``}\alpha) =_8 ((\mathrm{T}\uparrow(\alpha\text{``}\alpha))\text{``}\alpha) =_8 ((\alpha\text{``}\alpha)\text{``}\alpha) =_{14} ((\alpha\text{``}\alpha),,\alpha) =_8 ((\mathrm{T}\uparrow(\alpha\text{``}\alpha)),,\alpha) =_8 (\mathrm{T},,\alpha). \quad (15)$$

$$(\mathrm{T}\text{``}(\mathrm{T}\text{``}\alpha)) =_{11a} ((\mathrm{T}\uparrow\alpha)\text{``}\mathrm{T}) =_8 \alpha. \quad (16)$$

$$(\mathrm{T}\text{``}(\beta\text{``}\alpha)) =_{11a} ((\mathrm{T}\uparrow\alpha)\text{``}\beta) =_8 (\alpha\text{``}\beta) =_8 ((\alpha\uparrow\mathrm{T})\text{``}\beta) =_{12a} (\alpha\uparrow(\mathrm{T}\text{``}\beta)) =_8 ((\alpha\uparrow(\mathrm{T}\text{``}\beta)),,\mathrm{T}) =_{11b} ((\mathrm{T}\text{``}\beta),,(\mathrm{T},,\alpha)). \quad (17)$$

Let $((\alpha\text{``}\alpha)\text{``}\alpha)$, of which various equivalent expressions are presented in (14) and (15), be called $\mathrm{N}\alpha$, in words the *gradual* ("degree-al") *negate* of α. Obviously $\mathrm{N}\alpha$ is $-m$-adic where α is m-adic. The following identities proceed respectively from (16) and (17) when we introduce the 'N' notation in lieu of its equivalents as of (15):

$$\mathrm{NN}\alpha = \alpha. \quad (18)$$

$$(\alpha\text{``}\beta) = \mathrm{N}(\beta\text{``}\alpha) = (\alpha\uparrow\mathrm{N}\beta) = (\mathrm{N}\beta,,\mathrm{N}\alpha). \quad (19a)$$

(18) and (19a), in turn, yield these:

$$(\alpha,,\beta) = \mathrm{N}(\beta,,\alpha) = (\mathrm{N}\beta\uparrow\alpha) = (\mathrm{N}\beta\text{``}\mathrm{N}\alpha). \quad (19b)$$

$$(\alpha\uparrow\beta) = (\alpha\text{``}\mathrm{N}\beta) = (\beta,,\mathrm{N}\alpha) = \mathrm{N}(\mathrm{N}\beta\uparrow\mathrm{N}\alpha). \quad (20)$$

There are exactly as many concepts of any negative degree $-m$ as there are of the corresponding positive degree m. For, let α and β be n-adic, so that $\mathrm{N}\alpha$ and $\mathrm{N}\beta$ are $-n$-adic. If $\mathrm{N}\alpha = \mathrm{N}\beta$, then $\mathrm{NN}\alpha = \mathrm{NN}\beta$, wherefore, by (18), $\alpha = \beta$. Hence, $\mathrm{N}\alpha$ and $\mathrm{N}\beta$ are distinct if α and β are distinct. There is thus a distinct $-n$-adic concept corresponding to every n-adic concept. Giving n the successive values m and $-m$, we infer that there is a distinct $-m$-adic concept for every m-adic one and vice versa, and hence that there are exactly as many $-m$-adic as m-adic concepts.

That (6) cannot be maintained in the general case, granted (7)–(12), is shown by this inference:

$$((\alpha\text{``}\beta)\uparrow\gamma) =_{19a} ((\alpha\uparrow\mathrm{N}\beta)\uparrow\gamma) =_6 (\alpha\uparrow(\mathrm{N}\beta\uparrow\gamma)) =_{19b} (\alpha\uparrow(\gamma,,\beta)). \quad (21)$$

Study of (1)–(3) shows that $((\alpha\text{``}\beta)\uparrow\gamma)$ is not in general $(\alpha\uparrow(\gamma,,\beta))$ even when only natural degrees are involved, i.e., even when α, β and γ are of natural degrees whose minimum is that of β.

Note that (7)–(20), and even the repudiated identities (6) and (21), become arithmetical truths when we reread '$\uparrow$', '``', ',,', 'T' and 'N' arithmetically as '$+$', '$-$', '$-$', '0' and '$-$', or as '$\times$', '$\div$', '$\div$', '1' and '$1 \div$ ', respectively.

The introduction of negative degree achieves these simplifications:

(a) By (20), $(\alpha\uparrow\beta)$ becomes definable as $(\alpha\text{``}\mathrm{N}\beta)$, i.e., as $(\alpha\text{``}((\beta\text{``}\beta)\text{``}\beta))$.

Since $(\alpha''\beta)$ and $-\alpha$ are derivable in turn from the binary function $(\alpha^*\beta)$ $[= -(\alpha''\beta)]$, just $(\alpha^*\beta)$ and the unitary function $I\alpha$ remain as primitive. $(\alpha,,\beta)$ becomes definable more simply than previously, viz., as $(N\beta\uparrow\alpha)$, in view of (19b).

(*b*) Previously separate principles coalesce; thus (9)–(12) become corollaries variously of one another in view of the definitions in (a).

(*c*) Though arbitrary supplementary interpretation of $(\alpha^*\beta)$, $(\alpha''\beta)$ and $(\alpha,,\beta)$ in terms of concepts of natural degree is adequate to achieving significance for all arguments, the thus added cases will behave so differently from the original ones as to obstruct unified treatment of the general case. Such discontinuity is to be expected so long as these functions are held to be $(m - n)$-adic where and only where $m \geqslant n$, but is avoided by recognizing negative degree.

Note added in reading proof: The concepts of negative degree prove to be interpretable as any entities which are in one-to-one correlation with the concepts of positive degree and are not concepts of natural degree.

[1] Quine, "Toward a Calculus of Concepts." Awaiting publication.

EXTENSIONS OF PARTIALLY ORDERED SETS

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The object of this note is to present the principal results of a Doctoral dissertation[2] now being revised for submission to a mathematical journal.

A set, K, of elements, $a, b, c, \ldots$, is *partially ordered*[3] by the relation $\subset$ if Postulates 1 and 2 are satisfied.

1. POSTULATE. $a \subset b$ and $b \subset c$ imply $a \subset c$.
2. POSTULATE. $a \subset a$.

We define new relations and operations and consider postulates for closure under these operations. The study of the consistency and independence of these postulates gives a classification of partially ordered sets with respect to the operations under which they are closed. Multiplicative systems, lattices[4] or structures[5] and Boolean algebras[6] are found in this classification.

If K is a given partially ordered set closed under specified operations, then a set, L, is an extension of K whenever the following conditions are satisfied.

a. L is a partially ordered set closed under specified operations which include the operations under which the closure of K is specified.

b. L contains a subset isomorphic with K such that not only the ordering relation but also the operations for which the closure of L was specified, whenever they may be performed in K, are preserved.

c. L is the smallest set satisfying a and b. That is: Every set satisfying a and b contains a subset isomorphic to L, where the isomorphism is of the type specified in b.

An extension can be characterized by specifying the operations under which K and those under which L shall be closed. It is evident, from c that if K is closed under all the operations specified for L, then K and L are isomorphic. Hence, the repeated application of a given extension gives nothing new. Furthermore, if an extension can be accomplished in a number of steps the result of so doing is the same as though the extension were performed in one step or by some second sequence of steps. Therefore, the extensions which we give can be used as building blocks in constructing new extensions in any manner that fancy dictates, without jeopardizing the uniqueness of the result.

The preferred method of making extensions is to work algebraically with the elements, operations and relations of the given set. We call such an extension an *absolute* extension. An absolute extension is characterized by the fact that the extension of a subsystem is necessarily isomorphic with the corresponding subsystem of the extension. A *relative* extension employs the calculus of classes and may require infinite sections. As a rule, a relative extension is possible whenever an absolute one is. We use a relative extension only when an absolute one is impossible. In no case have we used any form of the axiom of choice.[7]

We need the following definitions.

3. DEFINITION. $a \supset b$ if $b \subset a$.

4. DEFINITION. $a = b$ if $a \subset b$ and $b \subset a$.

The set K is partially ordered by the relations $\supset$ and $=$. Hence, to every statement involving $\subset$ there are corresponding statements involving $\supset$ and $=$. In general, the statements involving $=$ are trivial, while those involving $\supset$ are significant and are called the *duals* of the original statements. In what follows we shall make statements for the relation $\subset$ and indicate in parentheses the changes needed to obtain the dual. A correspondence, $a \longleftrightarrow \bar{a}$, mapping a set into itself is a *dual automorphism* if it is one to one and if $a \subset b$ implies $\bar{a} \supset \bar{b}$ and conversely. We shall denote subsets of K with capital letters and elements of a subset with the same small letter and a subscript. Thus a_i is an element of A, and a_{ij}, of A_i.

5. DEFINITION. a is a *product*, Πa_i, (*sum*, Σa_i,) of A if $a \subset a_i$ ($a \supset a_i$)

for every a_i in A and $x \subset a_i$ ($x \supset a_i$) for every a_i in A implies $x \subset a$ ($x \supset a$).

6. DEFINITION. Πb_j (Σb_j) *distributes* b with respect to Πa_i (Σa_i) if $b = \Pi b_j$ ($b = \Sigma b_j$) and, for each b_j, a_i exists such that $a_i \subset b_j$ ($a_i \supset b_j$).

7. DEFINITION. ab ($a + b$) is *modular* with respect to a if every element, x, such that $ab \subset x \subset a$ ($a + b \supset x \supset a$) can be distributed with respect to ab ($a + b$).

8. DEFINITION. Πa_i (Σa_i) is *distributive* if every element, x, such that $\Pi a_i \subset x$ ($\Sigma a_i \supset x$) can be distributed with respect to Πa_i (Σa_i).

9. DEFINITION. O (I) is a *zero* (*one*) of K if $O \subset a$ ($I \supset a$) for every a in K. Collectively, O and I are called *units* of K.

10. DEFINITION. a' (a^*) is a *product complement* (*sum complement*) of a if $aa' = O$ ($a + a^* = I$) and $ax = O$ ($a + x = I$) implies $x \subset a'$ ($x \supset a^*$).

Elementary properties, such as uniqueness, the rules for equality, and the commutative, associative, distributive and idempotent laws for addition and multiplication, follow from these definitions.

Closure of a given set, K, under these operations does not follow from the definitions. Thus we are led to consider the following postulates.

11. POSTULATE. If a and b are in K, then ab ($a + b$) is in K.

12. POSTULATE. If ab ($a + b$) is in K, it is modular with respect to a.

13. POSTULATE. If ab ($a + b$) is in K, it is distributive.

14. POSTULATE. K contains a unit O (I), that is, the product (sum) of all its elements.

15. POSTULATE. If A is a subset of K, then Πa_i (Σa_i) is in K.

16. POSTULATE. If Πa_i (Σa_i) is in K, it is distributive.

17. POSTULATE. If a is in K, then a' (a^*) is in K.

18. POSTULATE. A dual automorphism exists in K.

19. POSTULATE. The correspondence $a \longleftrightarrow a'$ ($a \longleftrightarrow a^*$) is a dual automorphism.

In general, the dual postulates are not equivalent to the originals, so we shall denote them by asterisks. The relation of *being a subset of* partially orders the subsets of any set of points in a manner such that all these postulates are satisfied. Except for Postulates 19 and 19*, all the dependences between subsets of these postulates have been established and a method given for constructing the examples necessary to establish independence in the remaining cases.

Thus partially ordered sets may be classified according to the postulates they satisfy. In particular, partially ordered sets satisfying 11 are multiplicative systems, those satisfying 11 and 11*, lattices[4] or structures;[5] those satisfying 11, 11* and 12, modular lattices or Dedekind structures; those satisfying 11, 11* and 13, distributive lattices or arithmetic struc-

tures; those satisfying 11, 17 and 19, Boolean algebras.[6] If 11 and 11* are replaced by 15 and 15* respectively, the classification is said to be *complete*. We show further that 1, 11, 17 and 19, without 2, are postulates for a Boolean algebra and that in this case, 19 may be replaced by $[a']' \subset a$.

The first extension of an arbitrary partially ordered set is the adjunction of units, when they are missing. This can be done directly and all operations are preserved.

The second extension imbeds an arbitrary partially ordered set with units, K, in a complete lattice, L. The elements of L are the cuts in K, as defined in 20, and the ordering relation for L is defined in 21.

20. DEFINITION. *A cut (A, B), of K consists of two subsets, A and B, of K which satisfy the following conditions:*

a. $a_i \subset b_j$ *for every* a_i *in* A *and every* b_j *in* B,

b. $a_i \subset x$ *for every* a_i *in* A *implies* x *in* B,

c. $y \subset b_j$ *for every* b_j *in* B *implies* y *in* A.

21. DEFINITION. $(A, B) \subset (C, D)$ *if every element of* A *is an element of* C.

22. THEOREM. *L is a complete lattice.*

23. THEOREM. *The correspondence $a \longleftrightarrow (A, \bar{A})$, where A and $\bar{A}$ contain all the elements which satisfy the relation $a_i \subset a \subset \bar{a}_j$, carries the elements of K into a subset of L preserving the relation $\subset$, units, sums and products.*

24. THEOREM. The correspondence $a \longleftrightarrow (A, \bar{A})$ preserves distributive sums, distributive products, sum complements and product complements.

25. THEOREM. *Every complete lattice with a subset isomorphic with K has a subset isomorphic with L.*

26. THEOREM. If K is a Boolean algebra, then L is a complete Boolean algebra.

In particular, the smallest multiplicative system containing K is obtained by considering the subset of L consisting of the correspondents of K elements and products of a finite number of these. This is a relative extension.

By the third extension, an arbitrary multiplicative system, K, can be imbedded in a distributive lattice, L. The elements and ordering relation are defined for L in 27 and 28.

27. DEFINITION. *The elements of L are the subsets of K.*

28. DEFINITION.—*$A \subset B$ if, for every a_i in A, $a_i = \sum_j a_i b_j$, where the sum is taken over every b_j in B, and $\sum_j a_i b_j$ is distributive.*

29. THEOREM. *L is a distributive lattice satisfying Postulates* 15* *and* 16*.

30. THEOREM. *The correspondence $a \longleftrightarrow A$, where A consists of the element a alone, carries the elements of K into a subset of L so as to preserve the relation $\subset$, units, products and distributive sums.*

31. THEOREM. *Every distributive lattice satisfying Postulates 15* and 16* and containing a subset isomorphic with K has a subset isomorphic with L.*

In particular, if it is not necessary that Postulates 15* and 16* hold in L, the smallest distributive lattice containing K is obtained by considering only finite subsets of K as elements of L. This is an absolute extension. A single relative extension combining the last two extensions can be defined as follows. K is an arbitrary partially ordered set with units. Defining the elements and relation $\subset$ of L as in 32 and 33, an extension to a distributive lattice satisfying 15* and 16* is obtained.

32. DEFINITION.[8] The elements of L are the subsets of K satisfying the following two conditions.

a. If a_i is in A, then every x such that $x \subset a_i$ is in A,

b. A contains the distributive sum, if it exist in K, of every subset of A.

33. DEFINITION. $A \subset B$, if every element of A is an element of B.

The first set of a cut is a particular instance of the class of sets defined in 32.

The fourth extension imbeds an arbitrary distributive lattice with units, K, in a Boolean algebra, L. It is made in two steps. First, K is imbedded in a multiplicative system, K', of a special type, then, the third extension is applied to K'. The resulting set, L, is the sought-for Boolean algebra. We now define the elements and relation $\subset$ for K'.

34. DEFINITION. *The elements of K' are the ordered pairs (a_i, a_j), of K elements.*

35. DEFINITION. $(a_i, a_j) \subset (b_i, b_j)$ *if* $a_i \subset a_j + b_i$ *and* $a_i b_j \subset a_j$.

36. THEOREM. *L is a Boolean algebra.*

37. THEOREM. *The correspondence $a \longleftrightarrow (a, O)$ between the elements of K and a subset of K' preserves the relation $\subset$, units, distributive sums, sum and product complements whenever $a' = a^*$, and distributive products as products.*

38. THEOREM. *Every complete Boolean algebra having a subset isomorphic with K has a subset isomorphic with L.*

If the extension from K' to L is restricted to finite sums, both steps of this extension can be combined in the following canonical form and no essentially simpler canonical form is possible. In either form, this extension is absolute.

39. DEFINITION.[8,9] The elements of L are the finite sequences $(I = a_0 \supset a_1 \supset a_2 - - - \supset a_{2n} \supset a_{2n+1} = O)$, of K elements.

40. DEFINITION. $(I = a_0 \supset a_1 - - \supset a_{2n+1} = O) \subset (I = b_0 \supset b_1 - - \supset b_{2m+1} = O)$ if $a_{2i-1}b_{2j} \subset a_{2i} + b_{2j+1}$ for $i = 1, 2, \ldots n$ and $j = 0, 1, 2, \ldots m$.

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[2] Harvard University, May, 1935.

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FURTHER STUDIES OF THE CAROTENOIDS OF TWO PACIFIC MARINE FISHES, FUNDULUS PARVIPINNIS AND HYPSYPOPS RUBICUNDA, AND OF A MARINE ANNELID, THORACOPHELIA SP.

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Since the publication of our previous experiments on the carotenoid pigments in fishes (Sumner and Fox, 1933, 1935a, 1935b), I have had an opportunity to reëxamine the carotenoid pigment of these species, check carefully our qualitative tests and obtain spectrometric data with the use of a Bausch and Lomb wave-length spectrometer. The pigment in the Pacific killifish *Fundulus parvipinnis* was found by us in our earlier work to belong to the xanthophyll group, while that of the marine annelid worm *Thoracophelia sp.* upon which the fishes were fed belonged to the carotene class. We acknowledged the aid of Dr. James H. C. Smith and Mr. Harold W. Milner, who confirmed our findings by spectroscopic examination. We mentioned, however, at the end of our last paper (1935b) the desirability of spectrometric work for confirming our conclusion that ingested hydrocarbon(s) of the carotene group could be converted by the fishes to alcohol(s) of the xanthophyll series.

In the last joint paper (1935b) we overlooked a publication by Virgin

and Klussmann (1932) in which the authors claim to have observed an increase in egg xanthophyll in hens fed carotene but no xanthophyll. A supposition that the hens effected a conversion of carotene into xanthophyll would be in direct opposition to the results following from the careful work of Palmer and colleagues (see Palmer 1922). Sullmann and Vischer (1934) found that the ingestion by fasting human subjects of egg yolk or extracts thereof (the xanthophyll being almost all unesterified) was followed by a rise not only of xanthophyll esters, but of unsaponifiable carotenoids (carotenes) in the serum. Since they were measuring merely the change in the *concentration* of carotenes in one constituent of the body (serum) no evidence could be given, in their experiments, for the conversion of xanthophyll into carotene, the rise in concentration of which may have been due to mobilization from a place of storage. While it is not quite inconceivable that, in our worm-fed fish, the xanthophyll might have been formed from colorless compounds present in the food, instead of being derived from the carotene present, nevertheless this would seem to be extremely unlikely, since no animal organism has yet been shown to synthesize carotenoids. (Verne's contention that certain crustaceans may possess endogenous astacene is criticized by Lederer (1935b).)

Furthermore, in our own experiments (Sumner and Fox, 1935b) the control *Fundulus*, fed upon carotenoid-free (i.e., containing merely the faintest traces of carotene) flesh of the California "halibut" (*Paralichthys californicus*) showed, unlike the worm-fed and garibaldi-fed animals, no increase in their xanthophyll content after more than 90 days.

Tests were repeated on the original ligroin[1] solution of pigments of *Thoracophelia*, whose tissues had been exhaustively extracted of their carotenoids in the manner previously described. The solution had been stored for some months in a dark bottle kept away from light. Tests were made also on the carotenoid material taken from fresh worms. One type of carotenoid only was detected in the worm extracts; it was a carotene (or a mixture of α- and β-carotenes) as shown by the following properties which distinguish them from xanthophylls including the otherwise very similar cryptoxanthin: when, following alcoholic hydrolysis, a ligroin extract was shaken with 95% or even nearly absolute methyl alcohol, the pigment remained quantitatively epiphasic; no pigment whatever was adsorbed by dry $CaCO_3$ from either ligroin or carbon disulfide solutions (always strictly free from any traces of alcohol). The average values of numerous measurements, all of which were quite close, of the maximum region of the spectroscopic absorption bands were: I, 512.6 mμ, II, 482.0 mμ (CS_2 solution), which are close to the values given for α-carotene (Zechmeister, 1934, Lederer 1935b).

Fundulus.—Tests were then made on the ligroin solutions of carotenoids from the *Fundulus* which were fed *Thoracophelia*, also from those fed the

chopped flesh of the brilliant orange fish, garibaldi. The pigment from both batches of *Fundulus* behaved similarly in all tests and measurements made. Always primarily epiphasic when shaken with ligroin and 90% CH_3OH, the pigment was hypophasic after sufficient treatment with warm alcoholic KOH, indicating the hydrolysis of a xanthophyll ester. Furthermore the pigment was quantitatively removed from ligroin by dry $CaCO_3$, forming a yellow ring near the top of the filter column. Carbon disulphide solutions of the pigment from (*a*) *Thoracophelia*-fed fish, and of that from (*b*) the garibaldi-fed fish gave nearly identical absorption bands, the average maxima from numerous readings being respectively: (*a*) I, 497.4 mμ; II, 467.2 mμ; (*b*) I, 500.3 mμ; II, 471.2 mμ. These facts and data would indicate strongly the presence of either taraxanthin or violaxanthin, whose absorption maxima in carbon disulphide closely approach these values (Zechmeister 1934, Lederer 1935b). Treatment of an ethereal solution of violaxanthin with concentrated hydrochloric acid is reported by the authorities cited above to give a blue color, such treatment of taraxanthin giving no color. The pigments from (hydrolyzed) extracts of both the worm-fed fish and the garibaldi-fed fish when dissolved in diethyl ether and shaken with concentrated HCl gave no color whatever. The pigment is believed therefore to be closely related to, if not identical with taraxanthin.

Garibaldi.—The question of what pigment or pigments are responsible for the strikingly brilliant orange color of this fish has been asked by many persons. While our first paper (1933) reports its xanthophyllic nature as shown by several chemical tests, it was desirable to repeat the various tests, and to examine the pigment spectroscopically, especially in the light of the information which came out of our feeding experiments (1935b).

A ligroin solution of the pigment extracted from the skin of a large specimen had been kept in the dark at temperatures at or below freezing for several months. This solution remained perfectly clear and had lost no color at all, as determined photometrically. The extract had not been hydrolyzed and the yellow pigment was at this stage almost completely epiphasic. Indeed the yellow material could even be extracted in considerable quantity from the chopped tissue by direct applications of ligroin. Parenthetically, it may be remarked that it has been an unvaried experience to find esterified xanthophylls from fishes far more stable chemically than the free, hydrolyzed compounds. The epiphasic character of a carotenoid generally indicates that it possesses the nature of a hydrocarbon (carotene or lycopene), an esterified alcohol (i.e., a xanthophyll ester) or an acidic carotenoid of the astacene-like group.

When an alcoholic solution of KOH was added to the ligroin–alcohol system containing the unhydrolyzed, epiphasic pigment, no pigment was transferred to the nether layer of alcohol as would have been the case if free astacene had been present.

After hydrolysis had been effected by subjecting the extract to the action of warm alcoholic KOH for several hours, finally allowing the system to stand at room temperatures overnight, the carotenoid was found to be quantitatively hypophasic, being transferred, immediately upon shaking, from the ligroin layer into 90% CH_3OH. Again astacene was proved to be absent,[2] because after acidifying the system with a little acetic acid and reshaking, all pigment still remained in the alcohol layer, whereas if astacene had been present it would have been transferred under these conditions to the ligroin layer. The hypophasic nature of the pigment after hydrolysis also, of course, suggested strongly that we were not dealing with a carotene. The absence of carotene and the classification of this pigment as a xanthophyll were finally definitely established by the chromographic adsorption test, and by spectroscopic examination. The $CaCO_3$ chromatogram of the hydrolyzed, alcohol-free dry ligroin solution of pigment consisted of a single bright yellow layer at the very top of the column. The absorption spectrum of the pigment in CS_2 gave two strong bands and a third faint shadow farther down in the violet. The averages of many readings were: (*a*) unhydrolyzed pigment, I, 499.9 mμ; II, 471.1 mμ; (*b*) hydrolyzed pigment, I, 499.9 mμ; II, 469.3 mμ; the third (faintly visible) shadow occurred at around 437–440 mμ.

These values agree almost exactly with the visible absorption bands given for two xanthophylls of the $C_{40}H_{56}O_4$ series, namely, taraxanthin and violaxanthin, whose maxima in CS_2 are as follows: taraxanthin, I, 501 mμ; II, 469 mμ; III, 441 mμ; violaxanthin, I, 500.0–501 mμ; II, 469 mμ; III, 441 mμ (Zechmeister 1934; Lederer 1935). It will be observed also that the two strong bands correspond closely with those obtained for *Fundulus*, whether fed worms or garibaldi.

None of the prepared oxidation-products of carotene, such as the oxycarotenes, carotenones, etc., described by Zechmeister (1935) and by Mayer (1935) are reported to have the collective chemical and physical properties which have been described for these worm- and fish-pigments.

The critical test offered for distinguishing between the very similar isomers, taraxanthin and violaxanthin, is the reaction with strong HCl. When their ethereal solutions were shaken with strong HCl, neither the *Fundulus* nor the *Hypsypops* carotenoid material gave any color reaction.

It is provisionally concluded, therefore, that the carotenoid present in both *Fundulus* and *Hypsypops* is, for the most part, if not entirely, an esterified xanthophyllic alcohol identical with or isomeric with taraxanthin, which has been reported to be present in other animals as well.[3]

Summary.—While the marine polychaete worm *Thoracophelia sp.* seems to possess exclusively carotene(s) as its carotenoid pigment, and while the garibaldi, or marine "goldfish" *Hypsypops rubincunda* yields, on the contrary, a single pigment of the xanthophyll group, the Pacific killifish

Fundulus parvipinnis, whether fed upon the worm or upon the chopped flesh of the garibaldi, stores in its tissues only a xanthophyll very similar to or perhaps identical with the xanthophyll of the garibaldi.

Chemical tests and spectroscopic examination indicate that the carotene in *Thoracophelia* is preponderantly α-carotene; similar tests and observations indicate that the xanthophyll of the two kinds of fish is almost or quite completely esterified with organic acids *in situ* and is either taraxanthin, or an isomer of this carotenoid from which the tests in this work failed to distinguish it.

[1] Ligroin from Eastman Kodak Co. (P 513; B. R. 70–90°C.) was used in all of this work.

[2] Lederer (1935a) reports the isolation of astacene from the red skins of two kinds of fish: the dorado or gilt-head, *Beryx decadactylus*, and the goldfish, *Carassius auratus*.

The older work of MacMunn (cited by Palmer, 1922) described a lipochrome in the skin of the goldfish *Carassius auratus* whose spectra strongly resembled those of lycopene. Weissberger and Bach (1932) reported briefly regarding the carotenoids of the goldfish. Their preliminary article would lead to the conclusion that, whether or not more than one pigment is present, one carotenoid possesses the acidic nature characteristic of astacene.

[3] Burkhardt, Heilbron, Jackson and Parry (1934) obtained from the liver-oil of the angler-fish a yellow pigment which showed the absorption maxima of taraxanthin, while Brockmann and Volker (1934) found a new taraxanthin-like pigment in the yellow feathers of canary birds. They give experimental evidence to support their conclusion that the taraxanthin ($C_{40}H_{56}O_4$) of the feathers was derived from lutein ($C_{40}H_{56}O_2$) in the food.

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COLOR CHANGES IN ELASMOBRANCHS

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Introduction.—The early work on the color changes in fishes was done exclusively on teleosts. The study of the chromatophoral activities in elasmobranchs dates back only a little over a decade. In 1921 Schaefer stated that Raja clavata and R. batis showed no alterations in tint when on white or on black backgrounds nor to faradic stimulation of the skin. Six years later, however, Weidenreich (1927) called attention to the remarkable circumstance that Raja oxyrhyncha when first taken from the sea had a light ventral surface, but after a day in an aquarium this surface became uniformly dark. By microscopic inspection it could be seen that in the light phase the melanophore pigment was concentrated, but that in the dark one it was dispersed. In 1933 I showed that of two specimens of Raja erinacea the one that was placed in an illuminated white-walled aquarium for eighteen hours became light brown and the other in a black-walled illuminated tank dark brown. On transferring each fish to the other tank the light fish became decidedly dark after nine hours in the black-walled tank and the dark one pinkish white after twelve hours in the white-walled receptacle. From these last two sets of records it is clear that color changes occur in at least some species of rays.

Meanwhile Lundstrom and Bard (1932) had demonstrated a striking change of tint in the dogfish, Mustelus canis, and had shown that the dark phase of this fish was induced by secretions from the pituitary organ. Its light phase was subsequently declared by Parker and Porter (1934) to be dependent upon nerves. The blanching of the skin in Mustelus could be induced not only through stimulating the concentrating melanophoral nerves by cutting them, but also by faradic stimulation (Parker, 1935a). It also appeared from subsequent work that the terminals of the melanophoral nerves excite the concentration of pigment in the integumentary color cells through the production of an oil-soluble neurohumor, a lipohumor (Parker, 1935b).

These investigations were carried on without knowledge of the work done by Young (1933) on the autonomic nervous system in elasmobranchs. After a very exhaustive study of this part of the selachian nervous system, Young was able to show, particularly in Scyllium, that there were no recurrent gray remi communicantes, connections over which the autonomic nerve-fibres to the chromatophores regularly pass. Moreover, section of spinal, trigeminal or facial nerves was followed by no color changes in the skin nor did the skin blanch to injections of adrenalin. Fragments of skin

when immersed in solutions of this reagent likewise did not change color. Young was therefore led to believe that nerves had no direct control over the melanophores in Scyllium.

In consequence of this diversity of views as to the mode of action of the chromatophores in various elasmobranchs, I undertook to repeat some of the earlier tests made by Parker and Porter on Mustelus and to study such other elasmobranch material as was available at Woods Hole. As a matter of fact, I worked almost exclusively on Squalus acanthias which is to be had in large numbers in this locality from about the middle of May till early July. My investigations were carried on at the Woods Hole Oceanographic Institution where ample facilities were afforded me. I am greatly indebted to Dr. Henry B. Bigelow, director of this Institution, and to his staff for their many courtesies. The work was done under a generous grant from the Milton Fund of Harvard University.

Observations.—Squalus acanthias Linn. is a small shark at most a meter in length. It is deep slate colored with a tendency toward brown and is commonly marked by a few oblong whitish spots on its sides. When it is first taken from the sea it is ordinarily quite dark in tint and would be judged in general as a fish of much more pronounced coloration than its common associate, the smooth dogfish, Mustelus canis.

Ten specimens of Squalus were transferred directly from the outside water of the laboratory to a large wooden tank about a meter deep, a meter wide and six meters long. The tank had white walls, was illuminated from above by strong electric lights, and was provided with a generous supply of running, well-aerated sea water. In about two days three of these fishes had become noticeably pale in tint, the other seven remaining dark. This state of affairs was maintained for six days after which the test was discontinued. Obviously Squalus tends to remain in the dark condition and changes from that state much less freely than Mustelus does. Almost all specimens of Mustelus blanch regularly and relatively quickly in a white-walled aquarium.

Individuals of Squalus acanthias, pale from a sojourn in a white-walled tank, when transferred to a black-walled aquarium invariably turn dark after having remained there for two to three days. The certainty of this change as contrasted with the uncertainty of the reverse one is a characteristic of this dark-tinted form.

To ascertain the possible effect of blood-borne neurohumors in the color reactions of Squalus transfers of blood were attempted. Two very dark spiny dogfishes were fully bled from the caudal artery and the blood thus collected was defibrinated. One cc. of this blood was injected subcutaneously into each of two light fishes. No effect was to be observed either locally or generally, though the fishes were kept under observation some six hours. In a second pair of pale spiny dogfishes 5 cc. of such defibrinated blood were

injected under the skin just ventral to the anterior dorsal spine. In ten minutes both fishes had turned noticeably dark as compared with control individuals with which they had been closely matched before the experiment. After forty minutes the two experimental fishes were strikingly dark and, though they were in a white-walled tank, they remained dark for some six hours after which the experiment was discontinued. Defibrinated blood from dark fishes had no effect on the tint of other dark fishes into which it was injected. Blood was also taken from two pale fishes, defibrinated, and injected to the amount of 5 cc. each into two pale fishes and other equal amounts into two dark ones. No change in tint was subsequently noticed in any of these individuals. Apparently the blood of dark fishes carries a neurohumor which when introduced in sufficient amount into a pale fish will induce it to darken. Such blood has no effect upon dark fishes nor has the blood from light ones any effect on either light or dark individuals.

These results are in very close agreement with those obtained by Lundstrom and Bard (1932) on Mustelus, and the discovery by these investigators that the dispersing neurohumor in this dogfish was produced in the pituitary complex led me next to experiment on this organ in Squalus. Following the procedure of these authors for Mustelus, I operated on three dark Squalus by opening the cranium through the roof of the mouth and freeing and subsequently sucking out from each fish the pituitary organ. The three operated fishes were then returned to a black-walled tank where they were kept under observation. During the next two days no change in tint was noticed. On the third day one of the three was observably paler than the normal controls and on the fourth day all three had blanched considerably. By this time the fishes had reached what proved to be their maximum paleness, for although they lived, one of them one day longer and the others two days longer, they did not increase in paleness over these periods. At no time were the operated fishes as pale as the palest normal fishes that had from time to time come to my notice, but it must be remembered that many normal spiny dogfishes do not become fully light under any circumstances.

To test the effect of pituitary extract on the melanophores of the spiny dogfish the pituitary complex was removed from a given spiny dogfish, ground up with 1.5 cc. of sterile sea water and 1 cc. of this mixture was injected subcutaneously into a pale Squalus. In 25 minutes this dogfish had become as dark as the darkest fishes in the tanks and remained so till the next day when it was killed. This experiment together with those on the removal of the pituitary complex leads me to believe that the dark color of Squalus, like that of Mustelus, is due to a pituitary neurohumor produced in this gland and carried by the blood to the integumentary melanophores as described for Mustelus by Lundstrom and Bard (1932).

The injection into Squalus of obstetrical pituitrin (Parke, Davis and

Company) was also followed by a darkening of the fish. When 1 cc. of obstetrical pituitrin was injected subcutaneously into a pale Squalus, which was then retained in a white-walled aquarium, the fish in about an hour became mottled pale and dark. In an hour and a half after the injection it was fully and completely dark. Six hours after this it had become noticeably lighter and the next day while still in the white-walled aquarium it had returned to its initial light tint. Adrenalin was by no means so effective in changing the color of Squalus as was pituitrin. An injection of 1 cc. of adrenalin (1 part of adrenalin in 1000 parts of solvent, Parke, Davis and Company) into a dark Squalus was followed by a slight blanching in about three quarters of an hour. This change, however, soon passed off. When 2 cc. of adrenalin were injected there was a more lasting effect and 4 cc. of this reagent brought on a blanching that persisted more than three hours. After five hours the Squalus that had received this very large dose of adrenaline was again dark. Eight cc. of adrenaline induced a marked blanching which lasted half a day. These relatively enormous doses of adrenalin followed by only slight melanophore effects demonstrated the relative insensitiveness of Squalus to this reagent.

The very moderate and decidedly uncertain pale phase of Squalus is apparently not dependent upon any neurohumor carried in the blood. It may be due either to nervous action, as in Mustelus (Parker and Porter, 1934; Parker, 1935a, 1935b), or merely to the absence of the dispersing pituitary neurohumors as Young (1935) has recently shown to be the case in the lamprey and as is likely for common frogs.

To test this question resort was had to nerve-cuttings in the pectoral and the dorsal fins of Squalus. Parker and Porter (1934) showed that when in Mustelus short incisions were made transverse to the fin rays and their accompanying nerves and of such an extent as to sever a number of these nerves, a pale band resulted which extended from the cut to approximately the edge of the fin. This band was shown to be due to the excessive stimulations of the concentrating melanophore fibres. When similar cuts were made in the fins of Squalus such bands were only very rarely seen and when they were visible they were at best very faint indeed. In fifteen specimens of Squalus acanthias the pectoral fins and often also the dorsal fins were cut as described and of these fifteen fishes only five showed any evidence whatever of fin bands. Of these five, four had what could be described as very faint bands, the fifth showing on one dorsal fin a really obvious light streak. This streak developed in about ten minutes after the cut had been made and was present in the fish about an hour later when in consequence of other tests the fish was killed. In the four other spiny dogfishes in which bands occurred these bands were always very faint, in a few so very faint that had the bands not been seen in other cases they would have been entirely overlooked. The fact that fin bands are few

and faint in Squalus is in strong contrast with the condition in Mustelus (Parker and Porter, 1934) where the bands are regularly produced and invariably pronounced. While some of these tests were being made on Squalus a few Mustelus were brought into the laboratory. Such specimens were used to check the work of the previous year (Parker and Porter, 1934) and it was interesting to note that when the fins of Mustelus were cut, characteristic bands developed within ten minutes in fishes swimming in the same tank in which the cut but bandless Squalus were. Evidently these two species, Mustelus canis and Squalus acanthias, are strikingly different in this respect.

Another means by which concentrating melanophore nerves were identified in Mustelus was through faradic stimulation. If the nerve-fibres supplying a given area in a fin of the smooth dogfish are stimulated by a faradic current, the pigment in the melanophores of that area becomes concentrated and the area blanches (Parker, 1935a). On applying this method to Squalus not the least trace of a band in any fin so treated could be discovered though the test was carried out on some eight fishes. Here again the contrast between Squalus and Mustelus is most striking. The demonstration of concentrating nerve-fibres in Mustelus by both cutting and faradic stimulation appears to fail almost completely in Squalus and leads to the conclusion that concentrating melanophoric nerve-fibres in the spiny dogfish are at best only poorly represented and may possible be entirely absent from certain individuals. It is indeed remarkable that two forms so closely related as Mustelus canis and Squalus acanthias should show such striking differences. These two species in this respect offered a good instance of the importance of systematic distinctions in reference to physiological responses. The differences that they show offer a possible explanation for the contrast between Young's results (1933), already alluded to, on the chromatophoral system in Scyllium and the outcome of the tests by Parker and Porter (1934) on Mustelus. The absence of pale bands after nerve severance and of gray rami communicantes as reported by Young for Scyllium shows that in this shark a reduction of the autonomic component of the chromatophoral system can be even more complete than in Squalus which, as already shown, is in strong contrast with Mustelus whose concentrating autonomic fibres are apparently fully and effectively present. It would be interesting to ascertain whether gray rami communicantes are present in Mustelus and partly or fully absent from Squalus.

In conclusion it may be stated that the melanophore system of Squalus depends for the activation of its dark phase upon a pituitary neurohumor carried in the blood. Its pale phase is almost if not fully independent of nerves and probably results from the absence of the pituitary neurohumor.

Summary.—1. Squalus acanthias sometimes changes from a dark tint

to a pale one in a white environment and sometimes does not. It always changes from pale to dark in a black environment. In general it is a dark fish as compared with Mustelus canis.

2. Defibrinated blood from a dark Squalus will darken a pale Squalus but it will not change a dark one. Defibrinated blood from a pale Squalus does not change either a pale one or a dark one.

3. Loss of the pituitary complex of Squalus is followed in time by blanching.

4. Injections of obstetrical pituitrin will darken Squalus. Large injections of adrenalin are required to blanch this fish.

5. The cutting of nerves in the fins of Squalus is only occasionally followed by the formation of pale bands as contrasted with what occurs in Mustelus.

6. Faradic stimulation of fin nerves in Squalus is not productive of pale bands.

7. The concentrating autonomic nerve-fibres must be greatly reduced in Squalus as compared with their condition in Mustelus. In fact, in certain individuals these fibres may be entirely absent as they appear regularly to be in Scyllium.

8. The melanophore pigment in Squalus is dispersed by a blood-borne pituitary neurohumor. It concentrates in all probability chiefly from the absence of this agent.

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EXPERIMENTAL AND CLINICAL STUDY OF COBRA VENOM AS AN ANALGESIC

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Introduction.—Reptiles were used empirically in the primitive medicine of the ancients. Eminent physicians of the Middle Ages used different portions of various snakes as medicinal agents. Thus, for instance, Moses Maimonides, the Osler of medieval Hebrew and Arabic medicine, states in his treatise entitled, *Pirqué Mosheh*,[1] that certain parts of poisonous snakes were useful in the treatment of leprosy and *cancer*.[2] Even in the old English dispensatories of modern times, reptiles, including serpents, are classified under materia medica as, for instance, in the fascinating *Pharmacopoeia Londinensis*[3] of William Salmon. Parenteral administration of snake venom for medicinal purposes, however, is an achievement of recent years. Venoms or poisons of various species of snakes have thus been introduced into therapeutics in largely an empirical way within the last decade or two. Thus *Crotalus* or rattlesnake venom has been empirically employed in the treatment of epilepsy by Mays,[4] Fackenheim,[5] Prévost,[6] Jenkins and Pendleton,[7] Thom[8] and others; and the venom of the moccasin, *Ancistrodon piscivorus*, has been recommended for treatment of certain hemorrhagic conditions.[9] The most interesting and important contributions regarding snake venom, however, are those which have lately been written in France on *cobra* venom, which has been therapeutically employed on a rational and experimental basis. Calmette,[10] Taguet and Monaelesser,[11] Laignel-Lavastine and Koressios[12] have reported remarkable effects produced by injections of cobra venom in cases of malignant tumor, particularly the relief of the severe pains so common among patients affected with such diseases. These carefully controlled scientific medical researches of French writers prompted the present investigation. The four-fold objective of the writer was (1) to study the general pharmacology and toxicology of cobra venom according to the latest technical methods; (2) to prepare and assay biologically a sterile solution of cobra venom for therapeutic use in human patients; (3) to carry out a carefully controlled clinical investigation in order to ascertain the action of cobra venom injections on patients affected with malignant tumors causing severe pains; and (4) to analyze the more intimate pharmacodynamic action of cobra venom.

Snake venoms are general protoplasmic poisons which, however, exert a specially toxic action on two physiological systems, the blood and central

nervous systems, some venoms affecting the blood and others, the nerve elements. The constituents of snake venoms producing blood changes are variously named hemorrhagins, hemolysins, cytotoxins, precipitins, agglutinins, etc., according to the effect they produce. That constituent of snake venom having a predilection for nerve tissue is usually spoken of as neurotoxin,[13] the chemical nature of which is still unknown. Cobra venom contains a neurotoxin *par excellence.*

The action of cobra venom as a general protoplasmic poison can easily be demonstrated on protozoa and other animalcules, and on blood cells. It has recently been even more strikingly demonstrated by the author's phytopharmacological studies on living seedlings. Such tests proved that solutions of 1:25,000, and of even greater dilution, definitely inhibited the growth of roots of *Lupinus albus* seedlings.[14] Inasmuch as the small therapeutic dosage employed in clinical practice does not appreciably affect the blood elements, the writer's attention was devoted chiefly to the pharmacological effect of cobra venom on the nervous system and on the vital organs—heart, lungs and kidneys.

Pharmacology and Toxicology.—Dried crystalline secretion, or, more accurately speaking, scales of venom from three species of cobra, *Naia naia, Naia haji* and *Naia tripudians,* were used in this investigation. The specimens were procured through the courtesy and generosity of the author's colleagues in India and Egypt, to whom a lasting gratitude is hereby expressed. The dried secretion was dissolved in physiological sodium chloride solution, 0.9 per cent, which was used as the starting point for animal and clinical experiments.

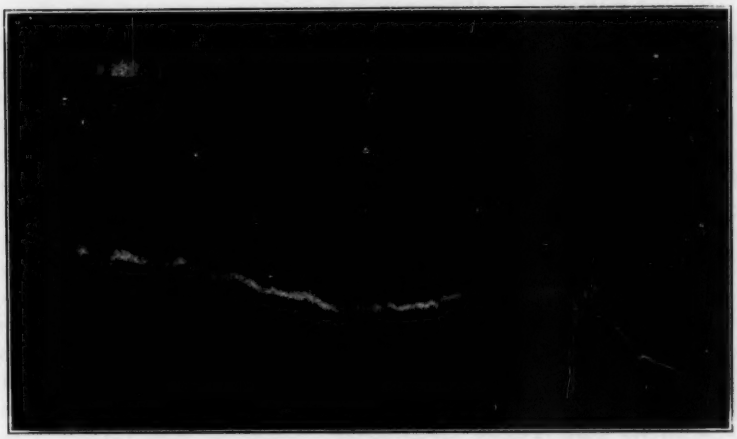
Zoopharmacological experiments were made on mice, rats, guinea pigs, rabbits, cats and dogs. The pharmacological action of dilute solutions of cobra venom on the respiration and circulation is best demonstrated by tests on cats or rabbits. The subjoined figure illustrates such an experiment. The carotid artery of a cat kept under ether anesthesia is cannulated and blood tracings are made while respiratory movements are recorded by a device which the author describes elsewhere.[15] A solution of cobra venom, 1:10,000, in physiological saline is slowly injected through cannula into the femoral vein at one-minute intervals. Such an experiment reveals that cobra venom first exerts a primary stimulating effect on both circulation and respiration. As the injections proceed, however, the respiratory center in the medulla is slowly depressed and finally paralyzed. In the last stages of medullary paralysis, the toxic effect on the heart is noted; but death occurs primarily through paralysis of the medulla, and the heart as a rule is brought to a standstill shortly afterward. Similar effects were produced in blood pressure experiments on rabbits and dogs.

The lethal dose of dilute solutions of cobra venom for cats, injected intravenously as described above, is 1.35 mg. per kilo weight, but varies

more or less according to the preparation tested. The lethal dose of the most potent preparation examined was 1.04 mg. while that of other solutions ran as high as 2.6 mg. per kilo weight of cat.

The Rowntree-Geraghty method[16] was used in studying the effect of cobra venom on the kidney function of rabbits intravenously injected with the drug. Injections of 5 mouse units in rabbits weighing 2 kilos produced no appreciable deterioration of kidney function.

The action of cobra venom was also studied by parenteral injection in guinea pigs, rats and mice. Mice afforded the most useful and economical means of assaying the venom. All that is necessary is to prepare a solution or colloidal suspension of the venom in physiological saline and to determine the minimal dose required to kill a mouse (20 to 22 gm.) within twenty



Effect of cobra venom (*Naia haji*), 1:10,000, on respiration and circulation of cat.

hours after intraperitoneal injection. This toxic dose is designated as a *mouse unit.* The mouse unit corresponds approximately to 0.01 mg. of venom, but some specimens give lower while others give higher ratings. For this reason it is not practical to express the therapeutic dosage of cobra venom in milligrams; the biological standard, expressed in mouse units, is usually employed instead.

The venom of the cobra is much more stable than that of other snakes, e.g., *Crotalus.* Whereas solutions of rattlesnake and moccasin venom deteriorate rapidly at room temperature, cobra venom decomposes but slowly below 70°C. This is a most fortunate circumstance when dealing with the preparation of a solution for use in human therapeutics. Higher temperatures and ultra-violet radiations, however, produce deterioration

of cobra venom solutions. The writer has described elsewhere the effect of light on cobra venom *in vitro* and *in vivo*.[17]

Preparation of Therapeutic Solutions.—Although solutions of cobra venom for laboratory use on animals and plants can be readily made, the preparation of a solution suitable for clinical administration is not a simple matter. The chief difficulty lies in securing a sterile solution free from bacteria and other microörganisms. After considerable experimentation, a method of preparing such a solution was developed. Dried scales of cobra venom were dissolved in physiological salt solution. The preparation was then put up in hard-glass ampules, sterilized by a special method at a temperature not exceeding 60°C., and assayed by the mouse method. Repeated tests proved that such ampules, when kept in the dark ice-chest, remained sterile and potent for many months.

Clinical Experiences.—Having succeeded in preparing a stable, sterile solution of cobra venom, the potency of which was accurately assayed by physiological methods, the writer in collaboration with several medical colleagues cautiously undertook a clinical study of cobra venom injections in a series of patients suffering from severe and uncontrollable pain. Such a clinical study could not have been successfully carried out had it not been for the keen interest and whole-hearted coöperation of two distinguished colleagues, Professor Joseph Colt Bloodgood,* of the Johns Hopkins Medical School, and Dr. Curtis F. Burnam, radiologist and surgeon of the Howard A. Kelly Hospital, Baltimore. The great majority of cases treated with cobra came to the writer through one or other of these colleagues and their associates. It was found that the average effective therapeutic dose of cobra venom for relieving pain in human beings was five mouse units. In a few cases a double quantity of the drug was injected at one time but in no instance was it necessary to give such large doses as those recommended by some French investigators. Injections were usually given intramuscularly and sometimes subcutaneously. In one case, intravenous injections were employed with no untoward effect. The usual therapeutic procedure was as follows: A dose of 2 or 3 mouse units was first administered to ascertain whether the patient had any idiosyncrasy to the drug. If no undesirable effect was noted, a full dose of 5 mouse units was injected the following day. The same amount was injected several successive days until a definite physiological effect (i.e., relief of pain) was noted. The dosage was then cut down in frequency, injections being given on alternate days. Later patients could be kept comfortable with one or two injections a week. In this respect cobra venom was strikingly different from morphine which usually leads rapidly to habituation requiring increasingly frequent dosage.

The majority of cases treated were patients suffering from carcinoma and other malignant tumors in which surgical operation had been contra-

indicated or proved to be unsuccessful in eradicating the lesion. Many of these patients had also been treated with deep x-rays and radium, and most of them had run the gamut of such pain-relieving drugs as antipyretics and narcotics. A few cases of intractable neuralgia and non-malignant diseases were also studied. Table 1 exhibits the variety and number of cases treated.

TABLE 1

PATHOLOGICAL CLASSIFICATION OF DISEASES TREATED

Cancer of breast	14	Cancer of ovary	4	Cancer of fallopian tubes	1
Cancer of cervix	14	Cancer of tongue	4	Cancer of intestine	1
Cancer of uterus	4	Cancer of floor of mouth	3	Cancer of larynx	1
Cancer of rectum	10	Cancer of glands (primary)	2	Myxolipoma	1
Cancer of jaw	8	Cancer of prostate	2	Cancer of orbit	1
Cancer of bladder	7	Cancer of spine	2	Cancer of penis	1
Cancer of retroperitoneal tissues	6	Cancer of thyroid	2	Cancer of stomach	1
Cancer of lungs and mediastinum	5	Cancer of tonsils	2	Arthritis	1
Cancer of bone	5	Cancer of antrum	1	Neuralgia	7
Epithelioma	4			Raynaud's disease	1

It will be noted that the largest number of cancer patients suffered from tumors of the uterine body or cervix. Next in number were tumors of the breast, cancers of the rectum, tumors of the lungs, cancer of the jaw, sarcoma of the bones and malignant diseases of the spine.

TABLE 2

SUMMARY OF CASES AND RESULTS

Total number of cases treated		115
Cases with incomplete histories		10
Number of cases analyzed		105
Doubtful results in	5 cases	4.7%
No relief in	9 cases	8.6%
Slight relief in	23 cases	21.9%
Definite relief in	30 cases	28.6%
Marked relief	38 cases	36.2%
		100.0%

Table 2 shows the results obtained in the 115 patients studied up to the time of this writing. Ten of the histories being incomplete were not included in an analysis of the therapeutic results, and 105 cases remained for analytic study. Of the 105 patients, nine obtained no relief and five cases gave doubtful results, which may be regarded as negative. Of the remaining patients, 23 cases, or 27.9 per cent, gave evidence of slight relief

after receiving several injections of cobra venom. Thirty cases, or 28.6 per cent, gave histories of definite and more lasting relief, while 38 cases, or 36.2 per cent, were markedly benefited. Not only was relief from pain effected in these patients but their general condition was improved and treatment with narcotics was temporarily suspended. If we disregard the 23 cases obtaining slight relief and add together only those patients who have obtained definite and marked relief, we find 64.8 per cent of them belonging to this group. If all the patients be counted, irrespective of the degree of relief they experienced, the percentage of more or less favorable results amounts to 86.7, as compared with 13.3 per cent or the group of patients in whom the results obtained were doubtful or negative. These figures compare very favorably with those given the writer by Professor A. Saenz of the Institut Pasteur in a personal communication in which he states, "Il a une réelle action anesthésiante dans 70% cas traités." Fortunately, not a single case under the care of the writer and his colleagues showed any toxic reaction after the cobra venom injections. This was not surprising, however, in view of the conservative dosage employed. Doses of 4 or 5 mouse units were followed by no untoward effect whatever when injected in the writer himself and his laboratory staff in connection with studies on the pain threshold which will presently be described. Even nausea was surprisingly absent in all but two or three cases and in only one instance did a patient state that his pains, instead of being relieved, became more acute after the injection of cobra venom. These clinical data, while not numerous, certainly seem to warrant further experimentation with the drug.

A powerful analgesic or pain-relieving effect was undoubtedly produced in a large number of cases. Such an analgesic action was noted in some of the most intractable conditions as, for instance, in malignant tumors of the jaw, spine and pelvic bones. A number of the patients treated were morphine addicts. In such cases the writer and his colleagues were able to reduce the amount of narcotic to a minimum by substituting cobra venom injections and in a few instances opiates were dispensed with temporarily.

Pharmacodynamic Analysis.—The pain-relieving or analgesic property of cobra venom is of great interest from a purely pharmacodynamic point of view. It has been the general impression of earlier writers that such an analgesia is due to a local action of cobra venom. This was a fallacious deduction from the fact that the bite of a cobra caused numbness of the affected member. When injected at one time, such a massive dose of cobra venom will undoubtedly act as general protoplasmic poison, producing local paralysis and necrosis. The writer's systematic pharmacological analysis of the effects of dilute solutions of cobra venom on various organs and tissues, and particularly on nerve tissues, however, throws an entirely different light on the action of cobra venom in relieving pain. In the first

place, dilute solutions of cobra venom, tested by standard procedures (such as the frog method, rabbit's eye method and "wheal" method) for their local anesthetic effect on sensory nerve endings, produce no local anesthesia. In the second place, similar negative results were obtained with applications of cobra venom to ascending or descending nerve fibres of the sciatic and pneumogastric nerves. In the third place, studies on isolated surviving smooth muscle preparations (such as those of the small intestine, uterus, vas deferens and bladder) revealed that even considerable doses of cobra venom, 5 to 10 mouse units, introduced into 50 cc. of Locke solution, in which the surviving organs were suspended, affected the myoneural junctions and nerve terminals in such segments either not at all or to but slight extent. This was evidenced by their subsequent normal response to parasympathetic or sympathetic drugs, respectively—pilocarpine, atropine, ergotoxin and epinephrine.

All the pharmacodynamic evidence which the writer has gathered from extensive and intensive experimentation point to the higher nerve centers in the cerebrum as the seat of the analgesic action affected by cobra venom. In support of this conclusion, the following experimental data are adduced:

(1) Experiments made on mice, rats and guinea pigs revealed that injections of cobra venom are antagonistic to the pharmacological action of such so-called "cerebral convulsants" as camphor. This anticonvulsant action of cobra venom may perhaps explain its alleged usefulness in the treatment of epilepsy. (2) Even small doses of cobra venom, injected in guinea pigs and rats, produced a definite antipyretic effect, as may be illustrated by the following protocol:

EXPERIMENT ON GUINEA PIG WEIGHING 200 GRAMS

9:54 A.M.	Temperature, 100.2°F.
9:56 A.M.	Injected 2 mouse units of cobra venom
10:35 A.M.	Temperature, 98.6°F. Slight depression
12:00 M.	Temperature, 100.0°F.

This fall in temperature, considered with other experimental data, seems to indicate an effect on the temperature-regulating center in the brain. (3) Extremely interesting findings regarding the cerebral action of cobra-venom injections were obtained in psychological experiments on the behavior of white rats trained in the circular maze, employed extensively by the writer for the study of the cerebral effects of various drugs and chemicals. Under the drive of hunger, white rats were trained to run from the periphery to the center of a circular maze. After the animals had learned to find their way to the dish of food placed in the center quickly and without making any mistake, they were injected with small doses of the cobra venom, and their running time, number of errors, neuromuscular co-

TABLE 3

THRESHOLD OF PAIN BEFORE AND AFTER INJECTION OF COBRA VENOM IN HUMAN SUBJECTS

SUBJECT	SOLUTION INJECTED	MOUSE UNITS INJECTED	THRESHOLD OF PAIN BEFORE INJECTION AT	THRESHOLD OF PAIN AFTER INJECTION AT						
C.K.	Naia naia	5	10:00 A.M.	10:23 A.M.	10:48 A.M.	11:10 A.M.	11:30 A.M.	12:30 A.M.	2:30 P.M.	4:30 P.M.
			5.0 cm.	4.0 cm.	4.5 cm.	4.4 cm.	4.1 cm.	4.3 cm.	4.2 cm.	4.8 cm.
M.M.	Naia naia	4	9:50 A.M.	10:10 A.M.	10:25 A.M.	10:45 A.M.	11:20 A.M.	11:45 A.M.	12:10 P.M.	12:30 P.M.
			5.0 cm.	4.4 cm.	4.2 cm.	4.1 cm.	3.8 cm.	4.0 cm.	4.2 cm.	4.4 cm.
S.P.	Naia haji	3	10:25 A.M.	10:45 A.M.	11:00 A.M.	11:15 A.M.	11:30 A.M.	12:00 M.	12:30 P.M.	3:40 P.M.
			5.0 cm.	4.3 cm.	4.0 cm.	4.0 cm.	3.0 cm.	4.0 cm.	4.5 cm.	4.9 cm.
R.M.	Naia haji	2	2:15 P.M.	2:30 P.M.	2:50 P.M.	3:00 P.M.	3:30 P.M.	4:00 P.M.	4:30 P.M.	4:45 P.M.
			5.9 cm.	5.4 cm.	5.3 cm.	5.0 cm.	5.2 cm.	5.0 cm.	5.4 cm.	5.5 cm.
N.G.	Naia haji	3	10:10 A.M.	10:35 A.M.	11:00 A.M.	11:40 A.M.	11:55 A.M.	12:30 P.M.	2:00 P.M.	3:30 P.M.
			5.5 cm.	5.2 cm.	5.0 cm.	5.0 cm.	5.0 cm.	5.6 cm.	5.0 cm.	5.3 cm
S.M.	Naia tripudians	3	10:28 A.M.	10:50 A.M.	11:05 A.M.	11:25 A.M.	11:48 A.M.	12:10 P.M.	1:00 P.M.	
			5.4 cm.	5.0 cm.	3.9 cm.	4.3 cm.	4.2 cm.	4.5 cm.	5.0 cm.	
H.B.	Naia tripudians	4	2:30 P.M.	2:53 P.M.	3:35 P.M.	3:55 P.M.	4:10 P.M.	4:34 P.M.	9:15 A.M.	11:45 A.M.
			7.1 cm.	6.5 cm.	6.6 cm.	6.4 cm.	6.4 cm.	6.5 cm.	6.8 cm.	7.0 cm.
M.M.	Naia tripudians	4	2:25 P.M.	2:55 P.M.	3:15 P.M.	3:35 P.M.	4:00 P.M.	4:35 P.M.	9:10 A.M.	
			6.0 cm.	5.0 cm.	5.2 cm.	5.0 cm.	4.9 cm.	5.2 cm.	6.2 cm.	
K.C.	Naia tripudians	4	2:30 P.M.	2:50 P.M.	3:35 P.M.	3:55 P.M.	4:15 P.M.	4:30 P.M.	10:00 A.M.	
			5.8 cm.	3.2 cm.	5.0 cm.	4.6 cm.	4.5 cm.	5.0 cm.	5.8 cm.	
D.M.	Naia tripudians	4	2:30 P.M.	2:45 P.M.	3:10 P.M.	3:35 P.M.	3:55 P.M.	4:20 P.M.	10:10 A.M.	
			6.5 cm.	4.8 cm.	6.2 cm.	5.2 cm.	5.3 cm.	5.5 cm.	6.5 cm.	
K.C.	Saline control—pH 4.6	2 cc.	2:15 P.M.	3:00 P.M.	3:35 P.M.	3:50 P.M.	4:10 P.M.			
			5.4 cm.	5.5 cm.	5.8 cm.	5.7 cm.	5.6 cm.			
B.M.	Saline control—pH 6.8	2 cc.	10:10 A.M.	10:35 A.M.	10:50 A.M.	11:10 A.M.	11:30 A.M.	12:00 M.	12:40 P.M.	
			4.9 cm.	4.9 cm.	5.0 cm.	5.0 cm.	5.0 cm.	5.0 cm.	5.0 cm.	

ordination and general behavior were studied. It was found that such a small dose as 0.5 mouse unit produced a primary stimulation of the cerebral and neuromuscular responses. The primary stimulation was followed by depression. The results of such experiments, described elsewhere in detail,[18] were strikingly similar to those obtained by Macht and Mora in studies on the effect of opium alkaloids.[19] The following protocols illustrate the findings:

Rat C, weighing 250 grams, injected with 0.5 mouse unit of cobra venom
Running time for three consecutive trips in maze:
(1) Just before injection: 31 seconds—no error
(2) 1 hr. after injection: 16 seconds—no error
(3) 2 hrs. after injection: 16 seconds—no error

Rat D, weighing 210 grams, injected with 3.0 mouse units of cobra venom
Running time for three consecutive trips in maze:
(1) Just before injection 18 seconds—no error
(2) 1 hr. after injection: stalled completely
(3) 13 hrs. after injection: 23 seconds—1 error

(4) Further and more direct evidence as to the central effect of cobra venom on relieving pain is furnished by the writer's experiments on the threshold of pain, as studied in both human beings and guinea pigs. In these tests, of which a detailed description is given elsewhere,[20] the threshold of pain marked on some specific point in the skin by the graduating induction current is determined before and after injection of the drug to be studied by a method detailed some years ago in these PROCEEDINGS in connection with a quantitative study of analgesia affected by opium alkaloids.[21] It was found that even as little as 3 mouse units of cobra venom, injected subcutaneously or intramuscularly in human beings, produced a definite depression of the pain threshold as compared with the result obtained in control experiments with injections of normal physiological saline solutions of different hydrogen-ion concentration. These results are strikingly illustrated by the subjoined table (table 3).

Discussion.—The results of the present investigation may be briefly restated as follows: A toxicological study of solutions of cobra venom in physiological saline reveals that lethal doses produce death primarily through a paralysis of vital centers in the medulla. Such paralysis is rapidly followed by arrest of the heart and fall of the blood pressure to dead level. The lethal dosage of the venom in great dilution injected continuously, while very small, is not as potent as that of some other powerful poisons which the writer has studied by similar methods. Thus, for instance, when tested by continuous intravenous injection in cats, cobra venom was usually found to be no more poisonous than nicotine and was certainly far less toxic than a solution of aconitin hydrochloride. The

potency of cobra venom for therapeutic administration is more conveniently expressed in mouse units than in milligrams. Standard solutions of cobra venom in saline were prepared for clinical use and sterilized by appropriate methods, and the average therapeutic dose of such a biologically standardized solution was found to be 5 mouse units, a dose which, if indicated, could be injected at regular intervals for long periods of time. Such solutions of cobra venom were employed in treatment of intractable pains of a series of 115 clinical cases, and extraordinarily favorable results, as far as relief of pain was concerned, were noted in a large percentage of the patients. This pain-relieving or analgesic effect of cobra venom on pharmacodynamic analysis seems to be a central one and points to the cerebrum as the seat of action. It is not a local anesthetic effect. When compared with the analgesia produced by morphine, the effects of cobra venom were found to supervene more slowly but proved, on the other hand, to be more lasting. While the action of cobra venom on the central nervous system in some respects is not unlike that of morphine, it differs from the latter in that it does not bring about the addiction and other undesirable features consequent upon injection of opiates and cocaine. The reason for the longer duration of the cobra venom effect is not quite clear but studies which the writer is making on the relation of this toxin and morphine to oxidative processes in brain tissue may help to throw more light on the phenomenon.

While the present paper is confined to a discussion of the analgesic or pain-relieving properties of cobra venom, it may not be irrelevant to mention that certain French investigators, notably Calmette, Saenz and Costil,[22] have found that cobra venom exerts a depressant or inhibitory effect on experimental tumors in mice. Such experiments are also being carried on by the writer but the data obtained so far are inconclusive.

Summary.—(1) An experimental study of cobra venom was undertaken with the four-fold objective (*a*) of determining its toxicological and therapeutic dosage, (*b*) or preparing a stable, sterile and biologically assayed solution for clinical administration, (*c*) of making a carefully controlled clinical study concerning the effect of injections of cobra venom in a series of cases suffering from intractable pain and (*d*) of ascertaining as far as possible its mechanism of action as a pain-relieving drug.

(2) A series of 115 clinical cases, treated with injections of cobra venom, revealed that such injections produced definite and marked relief of pain in nearly 65 per cent of the patients and a slight relief in even a larger number.

(3) Pharmacodynamic analysis of cobra venom action points to the higher nerve centers as the seat of the analgesia, resembling in this respect the action of morphine but differing from the opiate in that it does not produce addiction and other disagreeable and dangerous by-effects.

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STRUCTURAL AGE DETERMINATION OF PIEDMONT INTRUSIVES IN MARYLAND

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Problems and Method.—Most of the intrusives of the Piedmont region of Maryland and Pennsylvania are considered to be pre-Cambrian by many workers.[1] Port Deposit granite and Baltimore gabbro are intrusive into the Glenarm series, which is also considered pre-Cambrian.

A direct age determination, based on intrusive relations, is not possible because the intrusives are separated from known Paleozoic sediments by a wide belt of rocks of the Glenarm series (Fig. 1). A gap of approximately 20–30 miles must be bridged by other means. Persistent structures which can be recognized over a large territory and which transect formational boundaries regardless of their trend serve the purpose. Two such structures were utilized: flow cleavage and fracture cleavage. Hitherto

the age of the intrusives has been determined by indirect correlation and by the degree of their metamorphism.

Cleavage planes were measured and their directions plotted on maps. Thin sections were prepared for comparisons of structures, and the inclusions within the intrusives were carefully studied. The senior author made a large number of field excursions over the territory recording flow and fracture cleavage, and measured a section along the Susquehanna River from Safe Harbor to Conowingo. The junior author studied the Port Deposit granite structures. He also accompanied the senior author on many excursions. The age determination outlined below is based on

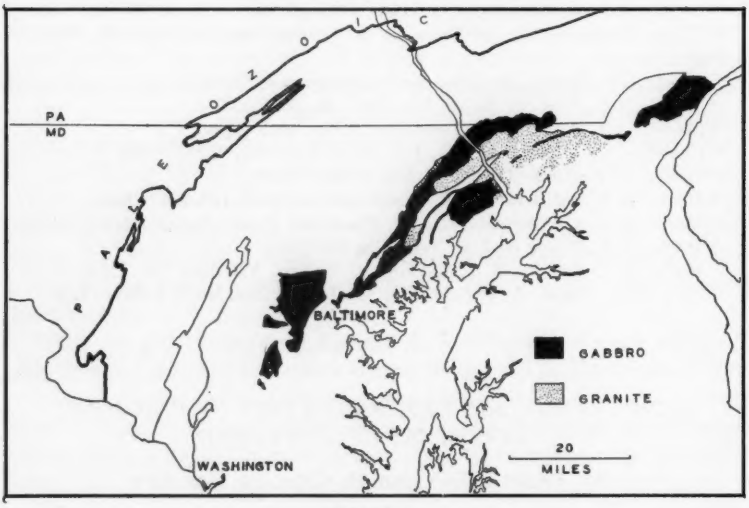

FIGURE 1

Distribution of Piedmont intrusives in Maryland (generalized). After: Md. Geol. Survey, 1933.

the correlation of Paleozoic structures in the northwestern portion of the region with those in inclusions within the intrusives in the southeast, and on the intrusive relationship between the igneous complex and the schist structures.

Separate detailed reports on cleavage, and on the structures of the Port Deposit granite will be published later.

Bedding, Flow Cleavage, Fracture Cleavage.—Bedding in the Glenarm series is obscured in many localities because of intense and probably repeated deformation. The lately renewed controversy regarding the age of the series hinges to a large extent on the recognition of bedding and on the question of whether or not Paleozoic and Glenarm beds are con-

formable.[2] Bedding can be observed in many localities, but not always with certainty. The question of bedding versus schistosity is not involved in this paper. Schistosity is always recognizable.

Both flow cleavage and fracture cleavage have been observed by all workers in a belt which parallels the Appalachians from Maine to Alabama.[3] Flow cleavage is used in this paper synonymously with schistosity, slaty cleavage and Schieferung;[4] fracture cleavage is used instead of false cleavage, slip cleavage and Schubklüftung. The relation between the two cleavages has been discussed recently by many authors.[5] A thorough discussion of the cleavage problem is beyond the scope of this study. These two cleavages are used because they are widely distributed and well known.

Flow cleavage can follow bedding or can transect it. In the area studied there is at least one cleavage which is accompanied by a thorough recrystallization and is regionally dominant. Quartz veinlets and granite dikes follow it. It is present in Paleozoic and Glenarm sediments and is conformable across their boundaries. Its direction is very persistent over large areas. Knopf and Jonas[6] ascribe it to a late period of deformation and suggest that it is late Paleozoic.

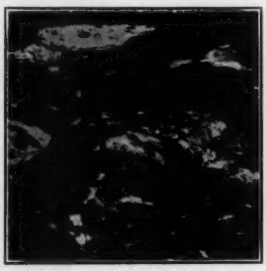

FIGURE 2

Oligoclase twin in Conestoga limestone (microphotograph). Scale: longest diameter of twin approx. 0.1 mm.

Albitization and replacement of older structures can be seen in the Wissahickon schist clearly following flow cleavage planes.[7] The same phenomena were found in the Conestoga limestone at Safe Harbor (Fig. 2). Oligoclase is very abundant here and follows certain layers parallel to bedding and flow cleavage planes.

Fracture cleavage transects flow cleavage (Figs. 3 and 4). The authors prefer the term fracture cleavage because recrystallization does not follow these planes. The deformation appears to be largely mechanical. Bedding and flow cleavage planes are displaced and in extreme cases highly crinkled. Older structures may be entirely obliterated. A new *s*-plane (Sandler[8]) is superimposed over an older *s*-plane.

Small quartz veins which carry a minor amount of feldspar follow this new plane. The authors believe they were formed very shortly after recrystallization and the formation of flow cleavage.

The direction of fracture cleavage is consistent over large regions. Its distribution can be studied in the literature, its constant direction can be seen in the map figure 5. The Martic overthrust and other disconformities produce no noticeable change in direction of the cleavage planes. The

Peach Bottom syncline, which presumably contains Paleozoic slate, is also transgressed by this new *s*-plane without regard for any boundaries. The only change in strike and dip occurs between the Wissahickon formation and the Peters Creek belt. Here the strike gradually changes from NE-SW to E-W, and the dip from vertical to gently north (Fig. 5).

Since both cleavages are found in Paleozoic rocks (Fig. 3) (Conestoga) and Glenarm series, and since their direction is not influenced by the contact between the two formations, and since albitization was found in both the Wissahickon schist and Conestoga limestone, the authors agree

FIGURE 3

Flow cleavage cut by fracture cleavage in Conestoga limestone (microphotograph). Natural size of section approx. 0.5 cm. A large proportion of the black mineral is graphite.

with Knopf and Jonas that the two cleavages were formed in post-Conestoga times and possibly during late Paleozoic periods of deformation.

Recognition within Gabbro and Granite.—Foliation in gabbro and granite parallels the flow cleavage of the Glenarm series. It is generally assumed that this is due to a post intrusive period of deformation. Its intensity, exemplified by the Port Deposit granite gneiss, has led to the conclusions that the gabbro and granites are pre-Cambrian.

Flow cleavage in the Glenarm series, however, is followed, transgressed and engulfed by granite and gabbro. Furthermore, it is destroyed by contact metamorphism and recrystallization. The writers believe, therefore, that the gneissic foliation is primary and was formed during the

TABLE 1

ROCK TYPES	STRUCTURES	INCLUSIONS	STRUCTURES OF INCLUSIONS	INCLUSIONS AND STRUCTURES WITHIN INCLUSIONS			
I. AMPHIBOLITE	Foliation						
II. SERPENTINE?							
III. GABBRO Massive Foliated	Foliation, Linear & Platy Joints	SCHIST	Bedding, Folds, Flow cleavage, Fracture cleavage				
IV. "DIORITE"	Foliation, Linear & Platy	AMPHIBOLITE					
		GABBRO Massive Foliated	 Foliation, Linear & Platy	SCHIST	Bedding, Folds, Flow cleavage, Fracture cleavage		
		SCHIST	Bedding, Folds, Flow cleavage, Fracture cleavage				
V. GRANITE (Port Deposit)	Foliation, Linear & Platy Joints Shear zones Marginal thrusts Gneissic borders	AMPHIBOLITE	Contact reaction or Sharp contacts				
		GABBRO Massive Foliated	 Foliation, Linear & Platy	SCHIST	Bedding, Folds, Flow cleavage, Fracture cleavage		
		"DIORITE"	Foliation, Linear & Platy	SCHIST	Bedding, Folds, Flow cleavage, Fracture cleavage		
		"DIORITE"	Foliation, Linear & Platy	GABBRO Massive Foliated	 Foliation, Linear & Platy	SCHIST	Bedding, Folds, Flow cleavage, Fracture cleavage
	Contact metamorphic; structures obscured along contact.	SCHIST	Bedding, Folds, Flow cleavage, Fracture cleavage				
		AUTOLITHS	Structure conformable with granite structure				
VI. DIKES	APLITE PEGMATITE HORNBLENDE-GRANITE MICRO-GRANITE	(May be in larger bodies)					
	BASIC DIKES	(May be all or in part Triassic)					

intrusion and consolidation of the magmas and after the cleavage was imposed on the sediments.

A detailed study of a large number of inclusions within the granite and gabbro was made and the results obtained are compiled in table 1. South of Conowingo Dam thousands of inclusions occur. Folded schists (Wissahickon and Peters Creek) with flow cleavage are engulfed by granite, quartz diorite and gabbro; and the internal structures of the inclusions are cut off at various angles by the contacts (Fig. 6). The inclusions are oriented parallel to the flow structure of the granite or quartz diorite.

FIGURE 4

Flow cleavage and bedding cut and displaced by fracture cleavage 6 miles north of Conowingo Dam, east side of Susquehanna River. Scale: white object $2^3/_4$ inches.

Though equiaxial fragments show random orientation, flat and elongated xenoliths show platy and linear structures.

Distinct fracture cleavage was observed in larger inclusions megascopically and under the microscope (Fig. 7*a*). The uniformity in strike and dip which is prominent outside of the granite contacts is lost in the inclusions. These were turned into odd positions by the intruding magma, were carried by it, and participated passively in its movements.

Contact metamorphism has destroyed flow and fracture cleavage in inclusions. In figures 7 *a–d* is a series of microphotographs beginning at the center of a large schist fragment and progressing toward a quartz diorite contact of that inclusion. The two cleavages are strong at the

center of the inclusion (Fig. 7*a*), but as the contact is approached they become gradually weaker and finally disappear (Figs. 7*a–c*). Parallel with this transition is an increase in newly formed minerals. Plagioclase,

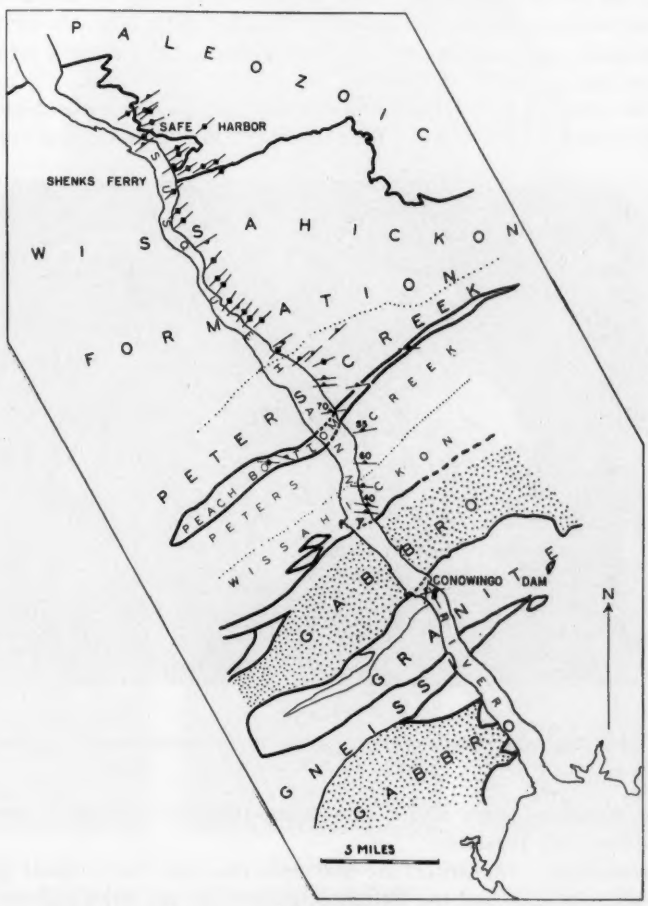

FIGURE 5.

Structure section along Susquehanna River from Safe Harbor to Conowingo Dam, showing constance of fracture cleavage.

entirely absent in the central portion, makes its appearance two or three inches from the contact (Fig. 7*d*) and increases greatly toward the contact. The unmetamorphosed center of the inclusion is a quartz phyllite composed

of chlorite, muscovite and quartz in about equal proportions. Small inclusions were altered completely by contact metamorphism.

If both flow structure in granite or quartz diorite, and cleavage in schist were due to the same causes, the cleavage would not be destroyed by contact metamorphism. Reactions of intruding magma on the structure of inclusions would not be possible. A condition which forms a structure cannot simultaneously destroy it.

Flow cleavage and fracture cleavage were formed after the deposition of the Conestoga limestone. The intrusives which have cut and engulfed

FIGURE 6

Schist inclusions in quartz diorite. 1/4 mile south of Conowingo Dam. Scale: white object 2 3/4 inches.

these structures must also be post-Conestoga and, therefore, not pre-Cambrian, but Paleozoic.

Conclusions.—The writers are convinced that the Port Deposit granite and its associates, and the Baltimore gabbro are not pre-Cambrian, but Paleozoic. Their age can be determined as post-Conestoga which has recently been assigned as post-Kinzers (lower Cambrian) age.[9] Any of the post-Cambrian orogenies may be responsible for the intrusions and the metamorphism of intrusives and sediments. Since the intrusions are part of a large belt of igneous activity it appears likely that other intrusives within this belt are also Paleozoic and not pre-Cambrian. Since Taconic deformation is little evident southeast of New York, it seems reasonable

FIGURE 7*a*

Flow and fracture cleavage in schist 40 inches from contact. Natural size of section approx. 0.5 cm.

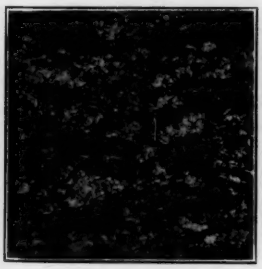

FIGURE 7*b*

Flow cleavage fading, fracture cleavage almost destroyed. Seven inches from contact. Natural size of section approx. 0.5 cm.

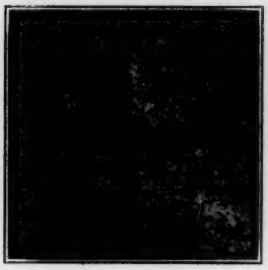

FIGURE 7*c*

Flow cleavage only in traces. Abundance of newly introduced minerals. Two inches from contact. Natural size of section approx. 0.5 cm.

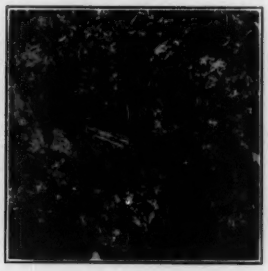

FIGURE 7*d*

Detail of 7*c*. Plagioclase in center. Natural size of section approx. 1 mm.

Figures 7*a–d*. Series of microphotographs of inclusion within quartz diorite south of Conowingo Dam.

to assume a late Paleozoic age for many of the Piedmont intrusives, possibly they have accompanied the Appalachian revolution?

Flow and fracture cleavage observations are possible in the entire Piedmont region. The cleavages are common and distinct, and the writers believe that a systematic investigation of them may shed more light on the age and the structure of many other intrusive masses in this province.

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